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Experience with a Quantitative Test for Normal or Decreased Amounts of Follicle Stimulating Hormone in the Urine in Endocrinological Diagnosis

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SINCE 1930 when Zondek (1) first described a method for the assay of follicle stimulating hormone (Prolan A, thylakentrin, pituitary gonadotropin A) in the urine, various modifications of this test have been in use in the Biological Laboratory of the Massachusetts General Hospital. The test has been invaluable, not only in the sorting out of the various causes for hypoestrinism (2), but also in the elucidation of various syndromes in males (3) as well as in females (4). Furthermore, the importance of the test is accentuated by the fact that follicle stimulating hormone is the only pituitary hormone at present that can be easily assayed. It is felt, moreover, that the failure of this test to gain widespread use in medical clinics is due to faulty methods.

In a previous communication (2) it was pointed out that hypoestrinism could be the result of primary ovarian failure or of ovarian hypofunction secondary to underproduction of follicle stimulating hormone. In the former condition, there is an increased production of follicle stimulating hormone, which results in increased amounts of it in the urine; in the latter condition, there is, of course, decreased production production of follicle stimulating hormone and presumably decreased excretion of it in the urine. Only three possible relation-

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ships between estrin and follicle stimulating hormone were recognized at that time: *a*) normal estrin production with normal production of follicle stimulating hormone; *b*) decreased estrin production with increased follicle stimulating hormone production; *c*) decreased estrin production with decreased follicle stimulating hormone production. Hence, in a patient with hypoestrinism, it was inferred that production of follicle stimulating hormone was decreased if its excretion was not increased. At that time, however, one could test only for increased excretion of follicle stimulating hormone. Now that one can test for its decreased-excretion (*vide infra*), this inference has been put to the test. The chief purpose of the present paper is to give the results of the tests for excretion of follicle stimulating hormone in various groups of endocrine cases in which *a priori* it was thought that the amount excreted should be decreased, and to point out a group of patients in which normal excretion levels were found when decreased levels had been expected. The causes of hypoestrinism will be tentatively reclassified.

METHODS

Obviously in any quantitative method, it is important to know the accuracy of the quantitations. In the various papers dealing with methods for concentrating the follicle stimulating hormone preparatory to assay, there has been an extraordinary lack of recovery experiments. To be sure, many authors have compared the results of one method with another, but Heller and Heller (5) and Varney and Koch (6) have been the only ones to make direct assays before concentration and to compare these with similar assays after concentration. Since in primary ovarian insufficiency there is a tremendous increase in the excretion of follicle stimulating hormone the assays both with and without concentration of the urine can be readily made. This, for a long time, has been the practice in our laboratory with each change in technique.

Heller and Heller (5) were the first to introduce a procedure which made it possible to quantitate follicle stimulating hormone in urine specimens which contained normal or subnormal amounts. Since a mouse can tolerate only 2.5 cc. of fluid (0.5 cc. at each of 5 injections) during the course of the test, one can, by injecting unconcentrated urine, test for only 40 mouse units (M.U.) per 100 cc. ($\frac{100}{2.5}=40$). By precipitating the hormone with ethyl alcohol it can be concentrated four times, and hence, one can test for 10 M.U. per 100 cc.; further concentration with alcohol is of no avail because the accompanying toxic substances kill the mice. Heller and Heller (5), by introducing a dialyzing procedure, have eliminated the toxic substances so that the hormone can now be concentrated to any desired degree.

It is, therefore, now feasible to test not only for increased levels of excretion of follicle stimulating hormone but also for normal or decreased excretion levels. In this clinic, urine specimens are tested for follicle stimulating hormone either by the 'non-dialysis' method or the 'dialysis' method. In the former, one tests for 96 and 192 M.U. per 24 hours; in the latter, for 6.6, and on certain occasions, for13 and 26 M.U. per 24 hours. The reasons these values have been chosen will appear below.

Technique of urine collections. Needless to say, it is important that the urine collections be accurate. The chief source of error arises from the fact that a patient will void and unintentionally throw out the specimen and then not remember that he has done so. Furthermore, another patient, who has made his collections correctly, in thinking back, will not be sure that he may not have missed one voiding. To obviate these difficulties, the use of the "overnight specimen" has been introduced. The patient voids on retiring, discards the urine and notes the time; any urine passed during the night is saved; on arising, patient voids, saves the specimen, and again notes the time. It is less likely that the patient will forget any voiding which occurred at night. Patients should be instructed to limit fluids in order to keep the total volume small.

Details of methods. The methods which have been finally derived and are now in use in this laboratory are in no way original. They merely represent slight modifications of other methods.[2] The authors would like to emphasize

[2] Although no real part of the present paper, the non-dialysis method, as now used is also included below. Small

especially that, in their experience, mice are more satisfactory test animals than rats and that precipitation with alcohol has proved more satisfactory than with tannic acid.

One minor modification introduced in this laboratory requires special comment. Dr. H. L. Fevold recommended, in a personal communication, the use of 8 instead of 4 volumes of alcohol for the precipitation of the hormone. In table 1 it will be seen that a slightly better yield is obtained in some cases with this modification Accordingly in the non-dialysis method, 8 volumes are used; in the dialysis method only 4 volumes are used as 8 volumes would entail the use of much alcohol for a very slight difference in yield.

TABLE 1. RECOVERIES OF FOLLICLE STIMULATING HORMONE USING 8 VOLUMES AS COMPARED WITH 4 VOLUMES OF ETHYL ALCOHOL

Patient	M.U.[1] of FSH/100 cc.			
	4 volumes		8 volumes	
	Greater than	Less than	Greater than	Less than
R.B.	50	80	50	80
R.B.	10	30	10	30
E.G.	10	30	30	50
H.S.	50	80	80	100
E.B.	50	80	80	100
M.P.	10	30	10	30
P.C.	80	100	80	100
K.	10	30	10	30

[1] Mouse units.

Non-dialysis Method

Preparation:

1. Take a 90-minute aliquot of a fresh overnight urine specimen; make acid to litmus and filter if cloudy.
2. Precipitate with 8 volumes of cold (ice-box) 95 per cent ethyl alcohol in a 1 or 2-liter Erlenmeyer flask. Let stand in ice-box overnight or until the supernatant is clear.
3. Siphon off most of supernatant and collect precipitate in a 50-cc. centrifuge tube, using some of the supernatant for transfer, and a 'policeman' if necessary.
4. Wash the precipitate twice with 15 cc. of absolute alcohol and once with 15 cc. of absolute ether; centrifuge each washing until the supernatant is clear. The second alcohol washing may remain cloudy after considerable centrifuging; a few cc. of absolute ether and an increased speed of the centrifuge hasten the complete clearing. Use the same glass stirring-rod throughout this and subsequent procedures.
5. Dry the precipitate in a vacuum desiccator overnight. The dried precipitate is stable and can be stored at room temperature until assayed.
6. On the day before the assay is begun, add 15 cc. of distilled water to the dry precipitate, mix thoroughly, and allow it to stand in ice-box overnight before dilutions are made.

Assay:

1. The criterion of a positive test is based on the weight of the mouse uterus. Our technique (7) is slightly modified from that employed by Levin and Tyndale (8). Two immature (*circa* 21 days) female white mice weighing 6 to 10 gm. are used at each dilution level. They are injected subcutaneously 5 times over a period of 3 days (twice the first day, twice the second, and once the third) with 0.5 cc., each mouse receiving a total of 2.5 cc. The mice are killed with illuminating gas 72 hours after the first injection. The uterus is considered enlarged if it weighs 7 mg. or more, after the fluid has been expressed by pressing between layers of filter paper. Uteri are weighed only when there is questionable enlargement.
2. Calculations: The final solution used for assay (15 cc.) represents the original 90-minute aliquot of urine. If 2.5 cc. gives a positive result, the 90-minute aliquot of urine contains at least 6 mouse units of follicle stimulating hormone or 1 unit for 15 minutes or 96 units per 24 hours.
3. Dilutions for testing for various levels of the hormone are made as follows and kept in the ice-box:

M. U./24 hr.	cc. of solution	cc. distilled H_2O
96	6.0	0.0
192	3.0	3.0
288	2.0	4.0
384	1.5	4.5
480	1.2	4.8

Dialysis Method

Preparation:

1. Take one or more fresh, overnight urine

improvements have been added here and there over a period of years by Dr. James A. Halsted. Dr. W. Beecher Scoville, Dr. Patricia H. Smith. Dr. Hirsh W. Sulkowitch, Elizabeth Cloney, Elizabeth Wallace Dodge, and the present authors.

specimens; make acid to litmus and filter if cloudy. The time of collection represented must be at least 8 hours if the test is to be made for 6.6 M. U. per 24 hours; 16 hours, if test is for 6.6, 13.2, 26.4, and 52.8 M. U. per 24 hours.

2. Add 1 gm. of NaCl for each 100 cc. of urine. Precipitate with 4 volumes of 95 per cent alcohol in a large Erlenmeyer flask. Let stand in ice-box overnight or until supernatant is clear.
3. Siphon off most of the supernatant and collect the precipitate in a 250-cc. centrifuge bottle, using some of the supernatant and a 'policeman' if necessary, for the transfer.
4. Wash the precipitate once with 50 to 60 cc. of absolute ether. Use the same glass stirring rod throughout this and subsequent procedures. Dry the precipitate overnight in vacuum desiccator.
5. Extract the precipitate 3 times with 10 to 15 cc. of distilled water each time. After thoroughly breaking up and mixing the precipitate, allow each extraction solution to stand for 30 minutes. Observations made in this laboratory show that 30 minutes is an adequate time for the hormone to go into watery solution. Centrifuge each extraction solution for 3 to 5 minutes, and pool them in a small Erleymeyer flask.
6. Transfer this solution (30 to 45 cc.), to a cellophane bag, $1\frac{1}{8}$ inches in diameter when inflated; use 2 to 3 cc. of distilled water to wash out the flask. Dialyze against running cold tapwater for 4 hours.
7. Re-precipitate the dialyzate, after adding 0.1 gm. of NaCl, with 4 volumes of 95 per cent alcohol, and let stand in the ice-box overnight or until the supernatant is clear.
8. Siphon off most of the supernatant and collect precipitate in a 50-cc. centrifuge tube, using some of the supernatant and a 'policeman' for transfer if necessary.
9. Wash the precipitate once with 15 to 20 cc. of absolute ether, and dry overnight in a vacuum desiccator. This precipitate is stable and can be stored at room temperature until assayed.
10. On the day before the assay is begun, add 5.5 cc. of distilled water to the dry precipitate of an 8-hour specimen or 11 cc. of distilled water to the dry precipitate of a 16-hour specimen; mix thoroughly and allow to stand in the ice-box overnight.

Assay:

1. The assay is made in the same way as that outlined for the non-dialysis method.
2. Calculations: The final solutions (5.5 or 11 cc.) represent the whole 8- or 16-hour urine collection, respectively. If 2.5 cc. give a positive result, the whole specimen contains at least 2.2 or 4.4 mouse units of follicle stimulating hormone respectively. If an 8-hour collection contains at least 2.2 mouse units, a 24-hour collection will contain at least 6.6 mouse units ($2.2 \times 24/8$). If a 16-hour collection contains at least 4.4 mouse units, a 24-hour collection will contain at least 6.6 mouse units ($4.4 \times 24/16$).
3. Dilutions: To test for 13.2, 26.4, 52.8 M. U. or higher amounts of follicle stimulating hormone, the necessary dilutions are made with distilled water. It is important that each mouse receive the same amount of fluid (2.5 cc.) and that solutions be kept in the ice-box between injections.

Recovery experiments. The recovery experiments consisted of two types: *a*) A comparison of assay of unconcentrated urine (direct method) with assay of the same urine concentrated by the non-dialysis method. *b*) A comparison of the assay of urine concentrated by the dialysis method with the same urine concentrated by the non-dialysis method.

To make the first comparison, the urine specimen must contain at least 40 M.U. per 100 cc.; to make the second, a urine specimen containing preferably at least 10 M.U. per 100 cc. should be used.

The following experiments (table 2) give some idea of the loss to be expected in using the non-dialysis method of concentration. With the exception of experiment 4 it will be noted that the recoveries were extraordinarily good. Although the data presented are rather meager,

TABLE 2. COMPARISON OF THE RECOVERY OF FOLLICLE STIMULATING HORMONE BY THE DIRECT METHOD AND THE NON-DIALYSIS METHOD

Experiment	Direct Method		Non-dialysis Method	
	M.U. of FSH/100 cc.		M.U. of FSH/100 cc.	
	Greater than	Less than	Greater than	Less than
1	100	150	100	150
2	40	100	50	80
3	40	67	50	80
4	40	67	10	30

previous recovery experiments (7) with essentially the same procedure have given similarly good recoveries.

In table 3 are shown the results of 16 experiments in which recovery by the dialysis method is compared with that by the non-dialysis method. It will be noted that the recovery varies from about 50 to 100 per cent. However, since concentration by the former method is of the order of magnitude of 40 as compared with 4 times in the latter method, these recoveries are surprisingly good. It has been determined that the loss occurs in dialysis since carrying out the procedure in exactly the same way without dialysis eliminates the loss. Studies are now in progress to determine what factors in dialysis cause the loss, and whether the percentage loss varies with the concentration of hormone.

TABLE 3. COMPARISON OF THE RECOVERY OF FOLLICLE STIMULATING HORMONE BY THE DIALYSIS AND THE NON-DIALYSIS METHOD

Experiment	Non-dialysis Method M.U./100 cc.		Dialysis Method M.U./100 cc.	
	Greater than	Less than	Greater than	Less than
1	10	20	5	10
2	10	20	10	20
3	30	50	16	27
4	30	50	30	50
5	30	50	11	22
6	50	80	16	32
7	50	80	10	30
8	100	150	50	80
9	50	80	80	100
10	100	150	80	100
11	80	100	10	30
12	10	30	5	10
13	10	20	5	10
14	5	10	5	10
15	10	30	10	20
16	10	20	10	20

Normal Values for Follicle Stimulating Hormone

Males. Specimens from 20 normal males were assayed for 6.6, 13, 26, 53 and 105 M.U. per 24 hours. Of the 33 tested specimens from these 20 males all were positive for 6.6 M.U.; 29 were positive for 13; 17 were positive for 26; 4 were positive for 53; and none was positive for 105 M.U. per 24 hours. It can be concluded, therefore, that normal post-pubertal males usually excrete more than 6.6 M.U. per 24 hours.

Females. Two 16-hour urine specimens were collected from each of 10 normal females at various times during the cycle, but with an interval of two weeks between the collections for each person. All 20 specimens were positive for 3 M.U. per 24 hours.

Twenty-five 16-hour urine collections from 15 individuals with normal menstrual cycles were assayed for 6.6, 13, 26 and 53 M.U. per 24 hours. The urines were collected during the first or last week of the menstrual cycle in an effort to avoid any peaks of excretion that might occur at the time of ovulation. All 25 individual specimens were positive for 6.6 M.U.; 24 were positive for 13; 11 were positive for 26; and none was positive for 53 M.U. per 24 hours. Two specimens were unintentionally obtained 11 and 13 days before the next period started, *i.e.*, around the supposed time of ovulation. These were positive for 26 and negative for 53 M.U. It can be conceded, therefore, that regularly menstruating females usually excrete more than 6.6 and less than 53 M.U. per 24 hours.

Follicle Stimulating Hormone Excretion in Abnormal Individuals

Assays were made on a series of patients in whom there was reason to believe that production and excretion of follicle stimulating hormone might be decreased.

Acromegaly. Cases 1 to 10 inclusive (table 4) suffered from acromegaly. *A priori* normal values might be expected for follicle stimulating hormone in those patients in whom the pituitary pathology had not destroyed the basophil cells, and decreased values in those in which it had. Furthermore, a female patient with acromegaly and with normal excretion levels of follicle stimulating hormone should develop an increased excretion level after the menopause. And, as a matter of fact, female acromegalics were found with decreased levels of follicle stimulating hormones, with normal amounts, and a rare case after the menopause, with an increased excretion level of follicle stimulating hormone.

Thus 7 patients (cases 1–7, inclusive) had excretion levels of less than 6.6 M.U. per 24 hours; of these 7, 5 were females and suffered from amenorrhea and 2 were males one of whom (case 4) had small gonads and loss of

TABLE 4. CLINICAL DATA AND FOLLICLE HORMONE EXCRETION LEVELS IN 10 CASES OF ACROMEGALY

Case and Hospital No.	Age yr.	Sex	Diagnosis	Clinical Data	FSH, Non-dialysis Method 10 M.U./100 cc.[1]	FSH, Dialysis M.U./24 hr.		
						Date	Greater than	Less than
1, 299114 (M.D).	43	F	Acromegaly; amenorrhea of FSH lack Nephrolithiasis	Classical acromegaly and amenorrhea when first seen 14 yr. ago; enlarged sella; visual fields normal; functionless left kidney with nephrolithiasis; serum phosphorus level, circa 4.5 mg./100 cc.; 17-ketosteroid excretion 15.3 mg./24 hr.; sugar curves showed slight hyperglycemia unresponsiveness and slight insulin insensitivity	Negative	3/13/42		6.6
2, 345658 (A.D.	32	F	Acromegaly; amenorrhea of FSH lack	Following the birth of her last child 5 yr. ago patient had a few periods followed by amenorrhea; no hot flashes; definite decrease in libido; noted change in odor of perspiration, increase in size of hands and feet and change in facial features. Typical acromegalic features; milk expressible from breasts; bitemporal hemianopsia; enlarged sella. Serum phosphorus 6.2 mg./100 cc. and 5.3 mg./100 cc.; 17-ketosteroid excretion 7.9 mg./24 hr.	Negative	4/ 1/42		6.6
3, 135659 (A.P.)	46	F	Acromegaly; amenorrhea of FSH lack ? hyperparathyroidism	Nursed youngest child 26 yrs. ago for 20 months; no periods since, no hot flashes; developed classical features of of acromegaly; passed uretral stone; x-rays showed enlarged sella and generalized decalcification; glucose tolerance test showed hyperglycemia unresponsiveness; 17-ketosteroid assays varied between 2.0 and 5.3 mg./24 hr.; serum calcium level high, circa 11.6 mg./100 cc. serum phosphorus not elevated as is rule in acromegaly and actually as low as 2.8 and 2.9 on two occasions; headaches relieved by x-ray therapy.	Negative	5/12/42		6.6
4, 344605 (J.M.)	41	M	Acromegaly	Acromegalic symptoms of 17 years' duration; marked visual difficulties; x-ray showed pituitary tumor; glucose tolerance test normal; serum phosphorus 4.0 mg./100 cc.; testes definitely atrophied; 17-ketosteroid excretion varied between 2.6 and 3.1 mg./24 hr.; insulin tolerance test showed insulin insensitivity; FSH test carried out shortly after subtotal resection of tumor.		3/28/42		6.6
5, 367869 (R.M.)	46	F	Acromegaly; Artificial menopause; Diabetes	Classical acromegaly of undetermined duration; 4 children, the youngest being 14 yr.; diabetes discovered 1 year previously; artificial menopause with radium 1½ years ago because of menorrhagia; hot flashes thereafter; marked impairment of vision of 1 year's duration; x-ray showed pituitary tumor; complete blindness of 1 eye and loss of temporal field on other side; no milk could be expressed from either breast; serum phsophorus level circa 5.2 mg./100 cc.; B.M.R. normal; 17-ketosteroid excretion 0.3 and 1.0 mg./24 hr.	Negative	12/18/42 1/14/43		13.0 6.6
6, 195410 (T.H.)	31	F	Acromegaly; amenorrhea of FSH lack	Classical acromegalic features; amenorrhea for 3 yr.; x-ray showed enlarged sella; visual fields normal; B.M.R. +7 and +38%; glucose tolerance test normal; 17-ketosteroid excretion varied between 3.6 and 6.0 mg./24 hr.; milk expressible from both breasts; serum phosphorus level, circa 4.8 mg./100 cc.		7/29/42 8/24/42 12/18/42	6.6	 6.6 6.6
7, 42654 (C.S.)	46	M	Acromegaly; Duodenal ulcer	Classical acromegaly of at least 22 years' duration; severe headaches; x-ray showed slightly enlarged sella; visual fields very slightly affected; glucose and insulin tolerance tests not remarkable; no loss of libido; testes normal in in size; 17-ketosteroid excretion between 8.2 and 13.0 mg./24 hr.; serum phosphorus 3.7 to 4.3 mg./100 cc.	Negative	5/18/42 11/14/42	6.6	 6.6
8, 347319 (C.S.)	18	F	Acromegaly; amenorrhea of FSH lack	Presented classical acromegaly of only a few years' duration; amenorrhea for 2 years; some hirsutism and acne; x-rays of skull showed enlarged sinuses but normal sella; visual fields normal; glucose tolerance test showed hyperglycemia unresponsiveness; insulin tolerance test showed insulin insensitivity; 17-ketosteroid excretion 7.7 and 8.4 mg./24 hr.; serum phosphorus level varied from 3.9 to 5.6 mg./100 cc.	Negative	4/25/42 6/19/42 7/15/42 9/25/42	6.6 6.6	 13.0 6.6 13.0
9, 349908 (P.K.)	38	M	Acromegaly	Classical acromegaly of 3 years' duration; severe left trigeminal nerve pain; loss of libido for 1 year; x-ray showed enlarged sella; visual fields normal; 17-ketosteroid excretion high, circa 22 mg./24 hr.; testicular biopsy showed normal spermatogenesis; serum phosphorus varied from 4.1 to 5.2 mg./100 cc.; glucose tolerance test normal.		10/19/42	6.6	24.0
10, 110968 (I.R.)	40	F	Acromegaly; physiological menopause	Pregnancy 9 years ago; unable to nurse child but milk expressible from breasts thereafter; developed classical acromegaly; gradual onset of menopause with hot flashes; x-ray showed enlarged sella; x-ray irradiation of pituitary relieved headaches; increased excretion of follicle-stimulating-hormone; 17-ketosteroid excretion 4.9 and 10.6 mg./24 hr.; serum phosphorus level circa 4.2 mg./100 cc.; B.M.R. slightly elevated, circa +18%.	Positive Negative Positive			

[1] The test using the non-dialysis method until recently was made for 10 M.U./100 cc. rather than on a 24-hr. basis.

libido. Two of these patients (cases 6 and 7), to be sure, had positive tests for 6.6 M.U. per 24 hours on one occasion and negative tests later on. Case 8, the youngest case in the series, was still excreting nearly normal amounts of follicle stimulating hormone. One male patient (case 9) gave positive tests for 6.6 M.U. per 24 hours. A testicular biopsy from this patient showed normal interstitial cells and spermatogenesis, which was confirmation of the normal function of follicle stimulating hormone. This was of considerable interest since the patient complained of loss of libido which was probably attributable to chronic pain and debility rather than to a lack of the hormone. Case 10 is included to show that some patients with acromegaly may produce increased amounts of follicle stimulating hormone after the menopause.

Panhypopituitarism. In table 5 are grouped together cases in which the diagnosis of panhypopituitarism was either made or considered. By the term panhypopituitarism is meant underfunction of all elements of the anterior pituitary. Simmonds cachexia represents an advanced degree of panhypopituitarism. The chief abnormalities in the results of the laboratory tests were a very low or absent 17-ketosteroid excretion (9), hypoglycemia unresponsiveness as shown in the insulin tolerance test (10), and a decreased excretion of follicle stimulating hormone.

In cases 11, 12 and 13, the panhypopituitarism directly followed a severe hemorrhage at the time of delivery, a common sequence of events as has been pointed out by Sheehan (11). Case 14 also had a severe hemorrhage at the time of a miscarriage, but the panhypopituitarism did not develop immediately thereafter. All 4 of these patients excreted less than 6.6 and 2 excreted less than 3 M.U. of follicle stimulating hormone per 24 hours; one of the patients who excreted more than 3 and less than 6.6 M.U. per 24 hours was receiving methyl testosterone therapy at the time of the test.

All 5 of the pituitary dwarfs (cases 15–19) had assays of less than 6.6 M.U. per 24 hours, and the 3 for whom assays were made for 3 M.U. were negative at this level. Unfortunately only in cases 16 and 17 were the tests made when the patients were not receiving either estrogen or testosterone therapy, two agents which are known to depress excretion of follicle stimulating hormone.

In cases 20, 21 and 22, the panhypopituitarism was caused by a non-functioning pituitary tumor or cyst. Cases 20 and 21 had follicle stimulating hormone assay levels of less than 6.6 M.U. per 24 hours; case 22 was first positive for 6.6 M.U. and later negative. In patients 23 and 24 the pituitaries were destroyed by suprasellar cysts; both patients had assay levels of less than 3.0 M.U. per 24 hours.

Thus, in cases 11 through 24, with clinical evidence of panhypopituitaries the tests showed diminished or absent excretion of follicle stimulating hormone. Cases 25, 26 and 27 are of special interest in that the results of the test ruled out the diagnosis of panhypopituitarism which had been made or at least considered. Case 25 in all probability had had panhypopituitarism due either to syphilis or to infarction of the pituitary following a hemorrhage at the time of delivery. However, at the time of the assay there had been a marked clinical improvement following anti-syphilitic therapy with a rise in the 17-ketosteroid excretion and a return of axillary hair. She excreted more than 26 and less than 52 M.U. of follicle stimulating hormone per 24 hours. Case 26 had certain findings which suggested panhypopituitarism, but this was ruled out by the test for follicle stimulating hormone which was positive for 6.6 M.U. for 24 hours, and the 17-ketosteroid excretion which was *circa* 3.5 mg. for 24 hours, and a histologically normal testicular biopsy. Case 27 was a patient at the Thorndike Clinic at the Boston City Hospital, whose urine was kindly furnished to us by Dr. Robert H. Williams.[3] The patient presented a difficult differential diagnosis between primary myxedema and panhypopituitarism. Until the test for follicle stimulating hormone was performed, the latter diagnosis was favored. In the authors' opinion, the excretion level of 26 M.U. of follicle stimulating hormone per 24 hours rules out this diagnosis. Case 28 is included to illustrate

[3] Cases 13, 21, and 27 were furnished to us by Dr. Robert H. Williams to whom the authors are very much indebted. Case histories have already been published (12).

TABLE 5. EXCRETION LEVELS OF FOLLICLE STIMULATING HORMONE IN 14 CASES OF PANHYPOPITUITARISM, IN 3 CASES IN WHICH PANHYPOPITUITARISM WAS CONSIDERED, AND ONE CASE OF MYXEDEMA

Case and Hospital No.	Age, yr.	Sex	Diagnosis	Clinical Data	FSH, Non-dialysis Method 10 M.U./100 cc.	FSH Dialysis M.U./24 hrs.		
						Date	Greater than	Less than
11, 160451 (M.W.)	38	F	Panhypopituitarism	Diagnosis first made in 1939; amenorrhea since profuse hemorrhage at second childbirth 9 yr. before; loss of axillary and pubic hair; marked anemia; B.M.R. −23%, cholesterol 367 mg.%; 17-ketosteroid excretion less than 0.5 mg./24 hr.; insulin tolerance test showed hypoglycemia unresponsiveness; x-ray of sella normal. Treated in 1941 and 1942 with methyl testosterone and thyroid therapy; cholesterol dropped from 510 mg./100 cc. to 176 mg.; first FSH test made while receiving methyl testosterone and thyroid, second while receiving estrin, methyl testosterone, and thyroid.	Negative	3/ 2/42 4/ 6/42	3.0	6.6
12, 177833 (H.S.)	33	F	Panhypopituitarism	Weakness, sensitivity to cold, dyspnea, amenorrhea, and loss of libido since second childbirth 7 yr. before first admission (1939); inversion of uterus and hemorrhage at time of delivery; appearance typical of myxedema; B.M.R. −40, −50%, cholesterol 112 mg.%; 17-ketosteroid excretion less than 0.5 mg./24 hr.; FSH excretion not increased; insulin tolerance test showed hypoglycemia unresponsiveness; hemoglobin 52 to 60%; electrocardiogram typical of myxedema; treatment with thyroid precipitated coma similar to Addisonian crisis with hypoglycemia (blood-sugar level 18 to 42 mg.%) and low serum Na (total base 134 m. eq./l.). At time of present FSH studies (1942) patient under the care of Dr. J. Lerman; markedly improved as a result of short period of treatment with pituitary gonadotropin, followed by thyroid and sodium chloride therapy; 17-ketosteroid excretion slightly higher, 1.0 mg./24 hr.	Negative	2/17/42 4/19/42	3.0	6.6
13, BCH-1047166 (H.O'N.)	42	F	Panhypopituitarism	Placenta praevia 7 years before admission with marked loss of blood; complete cessation of periods thereafter; treated with thyroid periodically for 10 yr. with restoration of B.M.R. to normal but with unsatisfactory clinical improvement; physical examination showed classical features of myxedema; eyebrows sparse, axillary and pubic hair absent; 17-ketosteroid excretion 0.3 mg./24 hr.; B.M.R. −43%, cholesterol 262 mg./100 cc. of serum; insulin tolerance test showed hypoglycemia unresponsiveness; patient responded well to testosterone propionate pellets, desoxycorticosterone acetate pellets and thyroid by mouth. FSH test made before treatment was started.	Negative	1/22/42		3.0
14, 366795 (S.A.)	38	F	Panhypopituitarism	Onset of amenorrhea without hot flashes 5 yr. before admission and 1 yr. after miscarriage; considerable loss of blood at time of miscarriage; weakness for 2 yr.; loss of axillary and pubic hair for 1 yr.; 15 lb. loss in weight; marked chloasma of face; B.M.R. −31 and −38%; serum cholesterol 172 mg./100 cc.; 17-ketosteroid excretion 1.1 and 1.5 mg./24 hr.		7/27/42		3.0
15, 139660 (J.C.)	24	F	Panhypopituitarism; dwarfism	Weight 60 lb.; ht. 49.6 in.; span considerably greater than height; growth not normal since 3 yr. old; weak, disliked cold, no sexual development; appearance that of a 10-yr.-old child; retarded bone age; normal sella turcica; B.M.R. −33%; cholesterol 146 mg./100 cc.; thyroid treatment without benefit; insulin tolerance test showed hypoglycemia unresponsiveness; 17-ketosteroid excretion less than 0.5 mg./24 hr.; FSH test made while receiving estrin therapy.	Negative	2/16/42 5/26/42 5/27/42		3.0 6.6 6.6
16, 48333 (C.D.)	16	F	Panhypopituitarism; dwarfism	Diagnosis of hypopituitarism first made at the age of 11; then measured 45.7 in., at 16 yr. 51.2 in.; no sexual development; sella turcica normal by x-ray, bone age markedly delayed; 17-ketosteroid excretion 0.0 to 0.9 mg./24 hr. when first seen; later, after estrin therapy, 1.3 to 2.5 mg./24 hr.; insulin tolerance test did not show complete hypoglycemia unresponsiveness; FSH tests made when off therapy.	Negative	3/21/42 6/30/42 7/25/42 10/ 1/42 2/ 6/43		6.6 3.0 3.0 3.0 3.0
17, 187779 (G.B.)	48	F	Panhypopituitarism; dwarfism	Height 54¾ in.; weight 80½ lb.; fragile-looking, prematurely-aged little woman with childlike voice and face; no breast development, no body hair; genitalia infantile; skin fine and atrophic; x-ray showed diffuse osteoporosis, ununited epiphyses of right knee and phalanges, slipped radial epiphysis and Osgood-Schlatter's disease; B.M.R. −6%; insulin tolerance test showed hypoglycemia unresponsiveness; first FSH test was made while receiving estrin therapy, second, after one month without therapy; 17-ketosteroid excretion 0.3 and 0.6 mg./24 hr.; cholesterol 216 and 323 mg./100 cc.	Negative	2/28/42 4/ 1/42		2.5 3.0

TABLE 5. EXCRETION LEVELS OF FOLLICLE STIMULATING HORMONE IN 14 CASES OF PANHYPOPITUITARISM, IN 3 CASES IN WHICH PANHYPOPITUITARISM WAS CONSIDERED, AND ONE CASE OF MYXEDEMA—*Continued*

Case and Hospital No.	Age, yr.	Sex	Diagnosis	Clinical Data	FSH, Non-dialysis Method 10 M.U./100 cc.	FSH Dialysis M.U./24 hrs.		
						Date	Greater than	Less than
18, 64011 (M.L.)	16	M	Panhypopituitarism; dwarfism	FSH test carried out while receiving 100 mg. of methyl testosterone weekly; height 55.0 in., weight 74½ lb.; under observation for 5 yr.; considered normal until age of 5; very little growth thereafter until testosterone therapy initiated; physical examination showed typical pituitary dwarf; had one episode of hypoglycemia after initiation of thyroid therapy; B.M.R. before thyroid therapy −20%; 17-ketosteroid excretion before therapy less than 0.5 mg./24 hr.		3/25/42		6.6
19, 199523 (D.A.)	21	M	Panhypopituitarism; dwarfism	At time of FSH determination, patient receiving 20 mg. of methyl testosterone daily; height 59.8 in.; part of which is to be attributed to previous testosterone therapy; marked delay in bone age; no secondary sex development; insulin tolerance test showed hypoglycemia unresponsiveness; 17-ketosteroid excretion less than 0.5 mg./24 hr. when not receiving therapy.		7/ 2/42		6.6
20, 156946 (F.F.)	37	F	Panhypopituitarism; pituitary tumor; hypo-insulin diabetes	First entry in 1933 at age of 28; symptoms suggested Simmonds' cachexia; amenorrhea, headaches, weakness, loss of 47 lb., hypertension, partial loss of axillary and pubic hair; B.M.R. −21 and −25%; x-rays showed intra-sellar tumor; marked improvement with x-ray therapy. Second entry in 1938 showed slight progression of the panhypopituitarism and the addition of hypo-insulin diabetes; endocrine deficiencies were those present in "Houssay-dog"; glucose tolerance test showed hypoglycemia unresponsiveness; further x-ray therapy with an improvement of hypopituitarism, and hence, accentuation of hypo-insulin diabetes. Third entry in 1942; 17-ketosteroid excretion then 1.2 mg./24 hr.; serum phosphorus 3.6 mg./100 cc.; FSH test here reported performed on this third admission, patient receiving only insulin therapy.	Negative	4/18/42		6.6
21, BCH 1047262 (P.G.)	46	M	Panhypopituitarism; pituitary tumor	Five years before admission had gradual onset of weakness, scanty beard, impotence, and increased sensitivity to cold; thyroid treatment without benefit; general appearance suggested myxedema; no hair on legs and only small amount on pubis and in axillae; testes and prostate small; B.M.R. −25%; serum sodium 127 m.eq./l, serum potassium 5 m.eq./l ; 17-ketosteroid excretion 2.5 mg./24 hr.; x-ray showed expansion and erosion of the sella turcica; patient responded to roentgenotherapy to the pituitary and to treatment with pellets of testosterone and desoxycorticosterone and thyroid by mouth. FSH test made while receiving therapy.	Negative	6/19/42		6.6
22, 68854 (F.H.)	53	M	Panhypopituitarism; chromophobe pituitary tumor	First entry in 1933 for a bitemporal hemianopsia; x-rays showed intra-sellar tumor; received x-ray therapy; progression of eye trouble stopped. In 1941, developed symptoms suggesting panhypopituitarism; 17-ketosteroid excretion 0.6 and 1.5 mg./24 hr.; B.M.R. −17%; insulin tolerance test showed hypoglycemia unresponsiveness; serum phosphorus 3.8 mg./100 cc.; axillary and pubic pubic hair scanty, prostate small; testicular biopsy showed arrested but not absent spermatocytogenesis and absence of Leydig cells; gained 8 lb. on testosterone therapy; FSH tests 3 wk. and 3 mo. after cessation of testosterone therapy.	Negative	3/23/42 5/ 2/42	6.6	 6.6
23, 83970 (T.O'R.)	23	M	Panhypopituitarism; craniopharyngioma; short stature	62.3 in. tall; 94 lb.; cranio-pharyngioma partially removed at 11 yr. because of visual disturbances and polydipsia; convulsions brought patient under observation 8 yr. later; then exhibited pituitary cachexia with stunted growth, sexual infantilism, and progeria; 17-ketosteroid excretion then less than 0.5 mg./24 hr.; cholesterol 163 mg./100 cc. of serum. Present studies made about 3.5 yr. later after patient had grown 6 in. as result of testosterone therapy; observations on FSH made 2.5 and 4 mo. following cessation of testosterone therapy; 17-ketosteroid excretion 5 mo. after testosterone therapy, 2.1 mg./24 hr	Negative	3/13/42 5/11/42		6.6 3.0
24, 354709 (L.S.)	19	M	Panhypopituitarism; craniopharyngioma; decreased stature	Increasing blindness led to the diagnosis of craniopharyngioma which was proved by operation; no secondary sexual development; testes very small; B.M.R. −37 and −42%; cholesterol 159 mg./100 cc. of serum; 17-ketosteroid excretion 1.1 and 1.3 mg./24 hr.; x-rays showed delayed bone age, epiphysitis of spine, and an intra-sellar tumor.		5/ 9/42 5/11/42		3.0 3.0

TABLE 5. EXCRETION LEVELS OF FOLLICLE STIMULATING HORMONE IN 14-CASES OF PANHYPOPITUITARISM, IN 3 CASES IN WHICH PANHYPOPITUITARISM WAS CONSIDERED, AND ONE CASE OF MYXEDEMA—*Continued*

Case and Hospital No.	Age, yr.	Sex	Diagnosis	Clinical Data	FSH, Non-dialysis Method 10 M.U./100 cc.	FSH Dialysis M.U./24 hrs.		
						Date	Greater than	Less than
25, 221187 (G.B.)	33	F	Panhypopituitarism with remission; syphilis	First entry in 1939; amenorrhea, weakness, nervousness, anemia and loss of axillary hair since birth of twins 5 yr. before; dry atrophic skin, scant body hair; insulin tolerance test showed hypoglycemia unresponsiveness; B.M.R. −4 and −9%; cholesterol 117 mg./100 cc. of serum; 17-ketosteroid excretion 1.3 and 1.5 mg./24 hr.; FSH excretion in urine not increased; Wassermann reaction positive. Received antisyphilitic and testosterone therapy; FSH studies here reported were made 1 to 3 mo. after cessation of all therapy; 17-ketosteroid excretion also higher, 2.3 to 3.4 mg./24 hr.; patient's condition paralleled laboratory tests—increased axillary and pubic hair, decreased atrophy of genitalia, although amenorrhea persisted.	Negative	5/25/42 7/20/42	26.0 26.0	 52.0
26, 288692 (M.B.)	55	M	Pituitary tumor; ? panhypopituitarism	Patient presented difficult diagnostic problem. In favor of panhypopituitarism were loss of libido and weakness, an intra-sellar tumor shown by x-ray, bitemporal hemianopsia, and hypoglycemia unresponsiveness as shown by the insulin tolerance test; against panhypopituitarism were the findings of normal axillary and pubic hair, normal prostate, 17-ketosteroid assays of 3.3 to 4.2 mg./24 hr.; and a failure to respond to methyl testosterone therapy. B.M.R. was −17%, serum cholesterol 128 mg./100 cc. The patient had pernicious anemia which was well controlled by liver therapy. A testicular biopsy showed normal spermatogenesis and normal Leydig cells.	Negative	3/ 7/42 4/ 1/42	6.6 6.6	
27, BCH 1058888 (E.D.)	60	F	? panhypopituitarism; ? myxedema	Eight years before admission, developed weakness, dizziness, sensitivity to cold, anorexia and abdominal cramps; presented characteristic appearance of myxedema, scanty pubic and axillary hair and thin eyebrows; nutrition relatively good; B.M.R. −33%; 17-ketosteroid excretion 1.4 mg./24 hr.; insulin tolerance test showed impaired hypoglycemia responsiveness; protein iodine 3 micrograms %; serum sodium 135.6 m.eq./l.; patient responded to treatment with testosterone, thyroid and desoxycorticosterone; FSH tests made before treatment.	Negative	2/22/42 3/19/42	3.0 26.0	
28, 350008 (R.A.)	50	M	myxedema	Classical myxedema of several years' duration, complicated by anemia and leukopenia. B.M.R. −38%; serum cholesterol 200 mg./100 cc.; 17-ketosteroid excretion 2.5 mg./24 hr.; electrocardiogram showed inverted T-waves, enlarged heart by x-ray. Made excellent clinical response to thyroid therapy.		4/15/42	6.6	

that the test for follicle stimulating hormone can be used to differentiate between primary myxedema and panhypopituitarism. This patient had unquestionable myxedema and a normal excretion level of follicle stimulating hormone.

Anorexia Nervosa. Before discussing anorexia nervosa a short digression on the effect of diet on the production of follicle stimulating hormone will be introduced. Werner (13) showed that starvation causes anestrus in rats, that the ovaries of such rats decrease in weight, that injections with gonadotropin restore the weights to normal, that the pituitaries of these starved rats have a decreased content of follicle stimulating hormone, and that starvation prevents the development of castration cells in the rat pituitary. The amenorrhea which young girls develop, when they take a very inadequate diet to improve their figures, is probably due to the effect of starvation on the production of follicle stimulating hormone; the fact that they do not develop amenorrhea if they are put on a very low caloric intake with adequate amounts of protein makes it probable that some amino acid is necessary for the elaboration of follicle stimulating hormone.

Deficiency of the Vitamin B complex also leads, in rats, to anestrus and atrophied ovaries; these ovaries are still responsive to follicle stimulating hormone (14, 15, 16). The anestrus in vitamin B deficiency in rats may likewise be due to an inadequate protein intake. Thus, Richter, Holt, Barelare and Hawkes (17) showed that rats on a vitamin B-deficient diet with respect to thiamin, riboflavin, nicotinic acid, B-6, and Castle's W factor choose a low carbohydrate, low protein, and high fat diet.[4]

In each of cases 29 to 33 (table 6) the diagnosis of anorexia nervosa with resulting amenor-

[4] From recent work by Figge and Allen (16b) it is quite clear that pantothenic acid is important in maintaining normal gonadotropic hormone production by the pituitary.

TABLE 6. FOLLICLE STIMULATING HORMONE EXCRETION LEVELS IN 5 CASES OF ANOREXIA NERVOSA OR QUESTIONED CASES OF ANOREXIA NERVOSA

Case and Hospital No.	Age, yr.	Sex	Diagnosis	Clinical Data	FSH Non-dialysis Method 10 M.U./100 cc.	FSH Dialysis M.U./24 hrs.		
						Date	Greater than	Less than
29, 363498 (E.S.)	16	F	Emaciation as result of faulty diet; ?anorexia nervosa; Von Recklinghausen's neurofibromatosis	FSH test made July, 1942; started reduction diet December, 1941, because her school friends teased her about her obesity; weight quickly dropped from 115 to 90 lb., and her menstrual periods ceased; weight 74¾ lb., height 63¾ in.; presented all the classical findings of neurofibromatosis: pigmented areas, subcutaneous fibromata, area of soft tissue swelling, bone lesions due to encroachment of nerve tumors; laboratory studies at the Pratt Diagnostic Clinic showed a basal metabolic rate of −42% and a 17-ketosteroid excretion of 3.8 mg./24 hr.; bone age by x-ray was not retarded.		7/ 8/42		6.6
30, 363341 (M.B.)	20	F	Malnutrition; ? anorexia nervosa	Went on "reduction diet" after graduating from high school because her friends called her fat; lost 60 lb. in 2 yr.; catamenia stopped 6 mo. after beginning of regime according to original history, but patient later stated that periods ceased while she was still eating an adequate diet; weight 60 lb., height 5 feet, pubic hair normal; B.M.R. −27%; cholesterol 159 mg./100 cc. of serum; glucose and insulin tolerance tests normal; 17-ketosteroid excretion 1.9 and 1.2 mg./24 hr.		7/ 1/42		6.6
31, 3563 (P.M.)	17	F	Anorexia nervosa	Catamenia started at 15 yr.; always irregular, every 2 to 3 mo.; amenorrhea for 9 mo.; loss of appetite for 7 mo.; frequent weeping and increased nervousness; 30 lb. loss in weight; x-ray of sella normal; insulin tolerance test showed decreased insulin sensitivity; 17-ketosteroid excretion 3.4 and 3.6 mg./24 hr.; B.M.R. −35% to −42%; 3 attempts at a 'medical D. and C.' without any flowing; patient receiving 10 mg. of progesterone daily for 5 days on last attempt rather than the usual 5 mg. a day for 5 days; at time when last FSH test was performed, patient was gaining.		5/18/42 7/ 7/42 1/18/43	6.6 26.0	13.0 6.6 53.0
32, 354589 (E.B.)	19	F	Anorexia nervosa	Went on "reducing diet" at 16 yr. and lost from 150 to 100 lb. in 3 yr.; developed anorexia during latter part of this period; difficult family situation; catamenia began at 11, regular at first, later irregular, finally no period for 7 mo. at time of FSH test; on physical examination, emaciated but had normal axillary and pubic hair; x-ray of sella normal; glucose tolerance test showed hyperglycemia responsiveness; 17-ketosteroid excretion 6.6 mg./24 hr.		6/ 8/42	13.0	26.0
33, 326850 (P.M.)	30	F	? anorexia nervosa	Long, complicated, psychiatric history; intermittent vomiting since second child was born 2 yr. ago; developed lactation during latter part of prolonged hospital stay; lost about 50 lb. and became very emaciated but later improved; 17-ketosteroid excretion at about low point of her debility was 1.8 mg./24 hr.; it later rose to 5.1 mg./24 hr. with an improvement in her general condition; first 'medical D. and C.' negative, second one, after 15 mg. of progesterone daily for 5 days, only slightly positive; last 3 FSH tests made after patient was considerably improved.	Negative	2/ 2/42 5/19/42 6/ 2/42 12/ 7/42 1/11/43	3.0 13.0 26.0 13.0	 6.6[1] 26.0 26.0

[1] This determination is probably incorrect as specimen stood at room temperature before being precipitated and became very alkaline.

rhea was at least under consideration. In this condition the cause of the amenorrhea has been thought to be a decreased production of follicle stimulating hormone resulting from inadequate protein in the diet. In cases 29 and 30 the assays were less than 6.6 M.U. per 24 hours, lending support to such an hypothesis. Although the first test on case 31 was positive for 6.6 and negative for 13 M.U., a second test was negative for 6.6 M.U.; 6 months later, after the patient had gained 10 lb. and was still amenorrheic, a third test was positive for 26 and negative for 53 M.U. Cases 32 and 33, however, had perfectly normal levels of follicle stimulating hormone excretion (positive for 13 and negative for 26 M.U.). These findings make one question the hypothesis that the amenorrhea is secondary to malnutrition in all of these cases, especially since one so often finds instances in which it seems quite clear that the amenorrhea preceded the loss of weight (case 31). The authors are rather inclined to believe that the amenorrhea in some of these cases has the same etiology as that in a group of cases which will be discussed below under the heading of amenorrhea with normal excretion levels of follicle stimulating hormone.

Idiopathic lack of follicle stimulating hormone

and amenorrhea. We have already discussed amenorrhea and lack of follicle stimulating hormone occurring in acromegaly, in panhypopituitarism and in malnutrition. Cases 34 and 35 in table 7 are examples of amenorrhea with lack of follicle stimulating hormone but without evidence of other pituitary diesease. These young women are both 21, have never had any periods, and exhibit almost complete lack of secondary sexual development. They are not dwarfed and have only a slight reduction in 17-ketosteroid excretion levels. The excretion levels for follicle stimulating hormone were less than 6.6 M.U. in both cases and in case 34 in which it was twice determined, the excretion of follicle stimulating hormone was less than 3 M.U. per 24 hours. Case 36, with rheumatoid arthritis and severe malnutrition, likewise had a decreased follicle stimulating hormone excretion without evidence of any very marked disturbance of the rest of the functions of the pituitary. She, of course, is an example of a very large group of patients with chronic disease and amenorrhea; in all probability the production of follicle stimulating hormone is very dependent on an adequate protein intake (*vide supra*) The amenorrheas associated with arrhenoblastoma are probably further examples of amenorrhea resulting from a lack of follicle stimulating hormone without other pituitary disease; unfortunately we do not have any observations on any proven case of arrhenoblastoma.

Amenorrhea with normal excretion levels of follicle stimulating hormone. The most interesting group in this study comprises those patients with definite hypoestrinism in which the excretion of follicle stimulating hormone was apparently in the normal range (cases 37–43 inclusive, table 7). In this group are

TABLE 7. FOLLICLE STIMULATING HORMONE EXCRETION IN 3 CASES OF IDIOPATHIC AMENORRHEA WITH LACK OF FSH AND 7 CASES OF AMENORRHEA WITH NORMAL LEVELS OF FSH

Case and Hospital No.	Age, yr.	Sex	Diagnosis	Clinical Data	FSH Non-dialysis Method 10 M.U./100 cc.	FSH Dialysis M.U./24 hr.		
						Date	Greater than	Less than
34, 229892 (C.W.)	21	F	Amenorrhea of FSH lack	First seen at 18 yr. of age; never had had periods, no sexual development; 68½ in., no breast development; axillary and pubic hair present, x-ray of sella normal; 17-ketosteroid excretion 6.9 and 7.4 mg./24 hr.; no excess of FSH in the urine. Increase of 17-ketosteroid excretion to circa 9.0 mg./24 hr. after prolonged treatment with estrin; FSH test here reported made after 5 wk. without estrin therapy.[1]	Negative	1/19/42 8/24/42		3.0 3.0
35, J.H.H. 285399 (J.O'B.)	21	F	Amenorrhea of FSH lack	Complete lack of secondary sex development; at 14 yr., pubic and axillary hair appeared. Ht. 65 in., span greater than height by 5 cm. Body, axillary and pubic hair normal. No breast development. Clitoris normal but vagina infantile. Peritoneoscopy showed normal sized, inactive ovaries, infantile uterus and tubes. Bone age retarded. X-ray of sella turcica normal. 17-ketosteroid excretion 4.4 and 5.8 mg./24 hr.[2]	Negative	3/ 5/43		6.6
36, 178032 (E.R.)	20	F	Amenorrhea of FSH lack; rheumatoid arthritis; extreme malnutrition	Patient bedridden with long-standing rheumatoid arthritis; amenorrhea of 8 months' duration; continuous fever; extreme malnutrition; marked wasting of the muscles; high sedimentation rate; 17-ketosteroid excretion 4.5 mg./24 hr.; low serum cholesterol, 95 and 100 mg./100 cc. of serum.		6/23/42		6.6
37, 306143 (B.C.)	19	F	Amenorrhea, with normal FSH level	Amenorrhea for one year while in nurses' training; two 'medical D. and C.'s' negative; B.M.R. −20 to −29%; thyroid therapy without benefit; cholesterol 140 mg./100 cc.; 17-ketosteroid excretion 10.6 mg./24 hr.; normal period after 3 weeks' vacation; diet normal; no hirsutism; sella turcica normal.	Negative	3/ 7/42	26.0	52.0
38, 322408 (I.H.)	22	F	Amenorrhea, with normal FSH level	Amenorrhea and lassitude 8 mo. in duration starting in Aug., 1941; came to Boston to dieticians' school in Sept., 1941, having had periods in Feb., April, June and Aug., 1941; 'medical D. and C.' with 20 mg. of progesterone negative; with 50 mg. of progesterone positive; B.M.R. −11%, cholesterol 179 mg./100 cc.; subsequently discharged on thyroid, 1 grain daily, and later 2 grains daily. After 3 mo. of such therapy, she started having regular periods which have persisted ever since (4 mo.).	Negative	4/16/42	13.0	26.0
39, 315067 (H.R.)	29	F	Amenorrhea, with normal FSH level	Periods regular until first pregnancy; pre-eclampsia and secondary anemia at time of delivery; amenorrhea since birth of child 6 years ago; fear of pregnancy and no libido or orgasms since delivery but normal libido before; slight obesity, but amenorrhea persisted in spite of thyroid medication; 'medical D. and C.' negative; 17-ketosteroid excretion 3.2 and 4.5 mg./24 hr.	Negative	1/28/42 3/30/42	3.0 13.0	26.0

TABLE 7. FOLLICLE STIMULATING HORMONE EXCRETION IN 3 CASES OF IDIOPATHIC AMENORRHEA WITH LACK OF FSH AND 7 CASES OF AMENORRHEA WITH NORMAL LEVELS OF FSH—*Continued*

Case and Hospital No.	Age, yr.	Sex	Diagnosis	Clinical Data	FSH Non-dialysis Method 10 M.U./100 cc.	FSH Dialysis M.U./24 hr.		
						Date	Greater than	Less than
10, 317690 (H.T.)	24	F	Amenorrhea, with normal FSH level	Amenorrhea since birth of only child 2 yr. ago; absolutely no lactation; periods began at 14, always irregular and late; never had orgasm; two 'medical D. and C.'s' negative; B.M.R. −18%; 17-ketosteroid excretion 5.4 mg./24 hr.; uterus responded normally to estrin therapy and estrin-withdrawal-bleeding was obtained; 'medical D. and C.' positive after 1 mo. of therapy with chorionic gonadotropin.	Negative	2/17/42 3/10/42 3/25/42	3.0 6.6 13.0	 26.0
11, 345653 (Y.B.)	30	F	Amenorrhea, with normal FSH level	Amenorrhea for 2½ yr.; onset following fifth pregnancy; persistent slight lactation; cramps at monthly intervals; D. & C. showed normal-sized uterus but complete atrophy of endometrium; decreased glucose tolerance; B.M.R.'s −16 to −26% but no improvement on thyroid therapy; cholesterol 166 mg./100 cc.; 17-ketosteroid excretion 5.9 and 8.5 mg./24 hr.; x-ray showed sella turcica normal; acromegaly ruled out on physical examination; serum phosphorus normal, 3.4 mg./100 cc. Patient had estrin-withdrawal-bleeding after 6 injections of estradiol benzoate at 6-day intervals indicating that her endometrium was not unresponsive.	Negative	5/ 4/42	13.0	26.0
42, 73066 (E.M.)	32	F	Amenorrhea with normal FSH level	Complained of fatigue and amenorrhea of 2 years' duration; periods became infrequent after birth of second child 9 years ago; endometrial biopsy directly before one of these periods showed secretory endometrium; two 'medical D. and C.'s' during first year of amenorrhea positive, a later one negative; no hot flashes at onset of amenorrhea, but diminishing libido for 4 to 5 years; B.M.R. −14%, thyroid treatment without benefit; x-ray showed sella normal; 17-ketosteroid excretions 4.3 and 7.1 mg./24 hr.	Negative	 6/ 9/42	6.6 13.0	13.0 26.0
43, PP	38	F	Amenorrhea, with normal FSH level	Amenorrhea for 7 years, preceded several years by normal pregnancy; onset of amenorrhea associated with gain of weight; was not relieved by loss of weight; diet not remarkable; no tissue obtainable for endometrial biopsy; thyroid medication without benefit; x-ray of sella turcica suggested pituitary tumor; x-ray irradiation of pituitary without benefit; 17-ketosteroid excretion 11.6 and 11.2 mg./24 hr.; more recent 17-ketosteroid excretion 5.0 mg./24 hr.; acromegaly ruled out on physical examination, and serum phosphorus only 3.3 mg./100 cc.; 'medical D. and C.' was negative; patient had extrin-withdrawal-bleeding after a course of estrin therapy.	Negative	4/15/42	13.0	26.0

[1] Exploratory laparotomy by Dr. Joe V. Meigs revealed infantile ovaries.
[2] The authors wish to thank Dr. R. W. TeLinde of Baltimore for permission to publish this case.

included case 37, a nurse, who developed amenorrhea when she started training and who had a menstrual period when she returned home; case 38, a student dietitian with amenorrhea for 8 months while in school; case 39, who has had amenorrhea since the birth of her only child 6 years ago; case 40, who has had amenorrhea for 2 years since the birth of her last child; case 41, who has always had irregular periods and who has had amenorrhea and persistent lactation and weakness without loss of weight since the birth of her last child 2.5 years ago; case 42, who has had diminishing libido for 4 to 5 years and amenorrhea and weakness for 2 years, and case 43, who has had amenorrhea for 3 years, associated with slight enlargement of the sella turcica. As evidence of complete lack of estrin production, 6 of these patients received 5.0 mg. of progesterone daily for 5 days and failed to menstruate thereafter. One patient, case 38, had a menstrual period after treatment with 50 mg. of progesterone. The other case had no endometrium at biopsy. In this clinic menstruation produced after giving a series of progesterone injections is termed a 'medical D. and C.'; absence of menstruation following such a procedure is termed a 'negative medical D. and C.,' and in the absence of a corpus luteum or pregnancy is evidence of lack of estrin production.

DISCUSSION

The discussion will be limited to a reclassification of hypoestrinism. The two types already predicated, *a*) primary ovarian insufficiency with an increased excretion of follicle stimulating hormone and *b*) a primary lack of follicle stimulating hormone with secondary ovarian insufficiency, definitely occur and need no further comment.

There seems to be a definite third group, however, characterized by a virtually complete lack of estrogen and yet a normal level of excretion of follicle stimulating hormone. Since Greep (18) has shown that pure follicle stimulating hormone will not produce estrogen except in the presence of small amounts of luteinizing hormone, one is tempted to predicate complete absence of luteinizing hormone in this group. Furthermore, since release of luteinizing hormone is probably under the control of the hypothalamus (*vide infra*), the trouble may be a failure of the hypothalamic-pituitary nervous pathways to release luteinizing hormone from the anterior pituitary. If this were the case, this condition could be well termed 'hypothalamic hypoestrinism.' Such an hypothesis might explain why so many of the patients in this group have complicating psychogenic factors (nurses away from home for the first time, immigrants, patients with sexual maladjustments and women with fear of pregnancy). Just why the lack of estrogen in this group does not lead to an increased production of follicle stimulating hormone is not clear. It is possible that in these cases follicle stimulating hormone is stimulating the ovary to produce some hormone which itself is non-estrogenic but which is a precursor of estrin and which has the property of inhibiting the production of follicle stimulating hormone.

To supplement the above discussion, it seems in order to summarize some of the evidence which suggests that the gonadotropic hormones are under the control of the hypothalamic-pituitary nervous pathway.

In the first place, there are probably three pituitary gonadotropins *a*) follicle-stimulating hormone (FSH), *b*) luteinizing hormone (LH), and *c*) luteotropin. FSH acts on the ovaries to cause ripening of the follicle; FSH plus a small amount of LH cause the follicle to produce estrin (*vide supra*); FSH plus LH cause ovulation; LH causes corpus luteum formation; finally, LH plus luteotropin causes the corpus luteum to produce progesterone (19).

In the normally menstruating woman the three gonadotropins are apparently set free more or less in sequence during each cycle without it being necessary that any special stimulus be brought to bear. In the cat and the rabbit, however, the sequence stops in the stage at which the follicles are ripe and producing estrin; for ovulation to occur, it is necessary that the animal have coitus, following which there is ovulation and the production of a functioning corpus luteum. Since it has been shown by Brooks (20) in rabbits that ovulation does not occur after coitus if the hypothalamic-pituitary nervous pathway is cut, it is highly probable that impulses over this pathway release the added amounts of LH necessary for ovulation. In the mouse and the rat the follicles develop to the stage at which corpora lutea are produced but do not function; if the animal has coitus during estrus, the corpora lutea function, *i.e.*, produce progesterone. This and other evidence (20) suggest that the stimulus arising from coitus causes an impulse to travel down the hypothalamic-pituitary nervous pathway which releases luteotropin. Since the above observations suggest that the release of LH and luteotropin are under the control of the hypothalamus in certain mammals it is not unlikely that such is the case in human beings.

A study of the phenomenon of puberty lends support to this supposition. Puberty is not due to the ovarian tissue becoming sensitive to gonadotropic hormones, since the ovaries of very young animals will respond to said hormones. Puberty is not due to the onset of production of gonadotropic hormones, since said hormones are already present in the pituitaries of very young animals. The possibility at least suggests itself that puberty is due to the release of gonadotropic hormones under the influence of nervous stimuli coming over the hypothalamic-pituitary nervous pathway. Such a thesis is supported by the findings in instances in which puberty fails to appear or in which it develops precociously. Thus, with suprasellar cysts interfering with the hypothalamic-pituitary nervous pathway, puberty may fail to develop (cf. Fröhlich's syndrome). Conversely, true precocious puberty (as opposed to the precocious production of one gonadal or gonadal-like hormone) is almost always, if not always, the result of some lesion in the hypothalamus, *i.e.*, a tumor or infection. A good example of true precocious puberty is that seen in a syndrome which has aroused much interest in this clinic (21, 22) and which is characterized by osteitis

fibrosa disseminata, areas of cafe-au-lait pigmentation of the skin, and precocious puberty when the condition occurs in females. Because of the considerations just discussed, it was predicated that the precocity part of this syndrome must be the result of an hypothalamic lesion; it is gratifying to be able to report that the first case in which the hypothalamus was studied at autopsy showed a definite lesion in one of the mamillary bodies.[5] For further discussion of the relationship of the hypothalamus to the pituitary gonadotopins, the reader is referred to the excellent article by Brooks (20). In the face of the evidence here cited, it is not surprising that situations would arise in the menstruating woman in which a disturbance in ovarian function was to be attributed to a disturbance in the hypothalamus.

It is of further interest that case 41 had persistent lactation. Selye, Collip and Thompson (23) showed in rats that suckling reflexes initiate the release of the lactogenic hormone from the hypophysis. Herold (24), quoted by Brooks (20), found that transection of the hypophyseal stalk prevents lactation. It seems very probable, therefore, that the hypothalamic-pituitary nervous pathway governs the release of prolactin and that a persistent lactation represents a disorder in the hypothalamus. Studies are under way to determine the nature of the amenorrhea which accompanies normal lactation.

Anorexia nervosa is a difficult diagnosis at best. The patient who tends to be obese, who becomes hypersensitive to obesity, who diets to extremes, and who eventually becomes paranoid about the whole subject is to be distinguished from the patient who has a psychopathic personality to begin with and who develops amenorrhea before the anorexia. In the authors' opinion, only the second of these patients has true anorexia nervosa. Probably either type of patient can develop a lack of follicle stimulating hormone due to faulty protein intake; however, it is suggested from the above data that the amenorrhea in true anorexia nervosa is initially due to lack of luteinizing hormone rather than to a lack of the follicle stimulating hormone.

[5] Personal communication from Edward H. MacMahon.

Summary and Interpretation

This study comprises an evaluation of the method (with minor modifications) of Heller and Heller for distinguishing between a normal and a decreased excretion level of follicle stimulating hormone.

All of a small group (35 individuals) of normal young males and females in the age group between 20 and 45 years excreted more than 6.6 mouse units of follicle stimulating hormone per 24 hours; only 4 of the 58 specimens examined were negative for 13.0 M.U. per 24 hours.

Ten patients with acromegaly were studied; these can be divided into 3 groups on the basis of the levels of follicle hormone excretion: *a*) Seven patients with decreased excretion of follicle stimulating hormone in which the tumor had presumably destroyed the basophil cells; *b*) 2 patients with normal excretion levels of follicle stimulating hormone in which the tumor had presumably not destroyed the basophil cells; *c*) 1 patient with an increased excretion level of follicle stimulating hormone in whom the tumor had presumably not destroyed the basophil cells and where the patient had passed the menopause.

Fourteen patients with panhypopituitarism had excretion levels of follicle stimulating hormone below 6.6 M.U. per 24 hours; in most instances (7 of 9) the level was also below 3.0 M.U. per 24 hours. In 3 cases in which the diagnosis of panhypopituitarism was considered, the excretion levels of follicle stimulating hormone were found to be normal and served as a basis for discarding the diagnosis of panhypopituitarism.

Two cases of primary amenorrhea were cited in which the excretion of follicle stimulating hormone was decreased and in which this was apparently the only abnormality in the pituitary function.

Nine cases were cited with clinical evidence of virtually complete lack of estrin production (as shown by a resting type of endometrium and by failure to menstruate following a 5-day course of progesterone therapy) in whom the excretion of follicle stimulating hormone was neither decreased nor increased; it is suggested that the primary abnormality in these cases may be a complete lack of release of luteinizing

hormone which lack in turn may be due to lack of stimulation of the pituitary over the hypothalamic-pituitary nervous pathway. The amenorrhea which occurs in some cases of anorexia nervosa before development of the anorexia may be explained on this basis; that which occurs later, after the malnutrition, is probably due to lack of production of follicle stimulating hormone resulting from faulty protein intake.

It would appear that with the tests for excretion levels of follicle stimulating hormone, one can divide cases of hypoestrinism into three categories: *a*) ovarian hypoestrinism due to primary ovarian insufficiency and associated with increased excretion of follicle stimulating hormone, *b*) pituitary hypoestrinism due to a primary lack of production of follicle stimulating hormone and associated with decreased excretion of it, and *c*) hypothalamic hypoestrinism due to disturbance in the hypothalamic-pituitary nervous pathway and associated with a normal excretion level of follicle stimulating hormone.

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Use of Desiccated Blood Pellets in the Biologic Assay of Blood Estrogens

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IN A recent publication, Albrieux (1) described a biological assay of blood estrogen by the intra-vaginal application of desiccated blood pellets. Previously several workers (2–5) had shown that cornification of the vagina of spayed rats can be produced with much smaller doses of estrogens applied directly to the mucosa than can be effected by parenteral administration. Lyons and Templeton (3) and Yerby (4) were able to produce cornification of the vaginal epithelium of spayed rats by the administration of 1/200 of a rat unit of estradiol benzoate. Albrieux (1) obtained a threshold reaction (induction of cornification although nucleated epithelial cells and leukocytes were still present) in 7 of 12 rats given 0.1 I.U. and in 11 of 12 rats given 0.2 I.U. of an estrone solution made from a sample of the International Standard, administered intravaginally in sesame oil. By administering an acetone-ether extract of whole blood intravaginally in sesame oil, Albrieux was able to demonstrate estrogenic activity equivalent to that of from 5 to 30 I.U. of estrone per 100 cc. of blood. In an attempt to improve this method of assay and to eliminate chemical extraction, Albrieux found that greater amounts of the hormone could be demonstrated by the use of pellets made from desiccated blood. The report presented here describes our experience with the latter method of assay.

METHOD

Throughout this work the techniques used by Albrieux were followed as closely as possible. For each test 15 cc. of blood was obtained from the subject by venapuncture and placed in a cellophane bag suspended in front of an electric fan in an incubator kept at 37° C. Complete desiccation occurred in a few hours. The dried blood was finely ground in a mortar and the resultant powder weighed to compare with the volume originally employed. We found that 1 cc. of whole blood dried by this procedure weighed an average of 215 mg. (±15 mg.). After the addition of 10 per cent of sesame oil the powder was compressed into pellets weighing from 30 to 90 mg. by means of a machine that was specially constructed to allow its use in a hydraulic press. The use of a constant amount of pressure resulted in pellets of uniform consistency; this type of pellet machine is considered an improvement over that described by other workers (6, 7), in which the pellets are compressed by manual pounding.

Rats of the Sprague-Dawley strain having normal estrous cycles were bilaterally ovariectomized at the age of 2.5 to 3 months, care being taken to excise a small portion of the uterus in order to insure complete removal of ovarian tissue. After a period of one week smears were taken daily for 7 to 10 days and only those animals showing a predominantly leukocytic smear over this period were selected for use. These rats were then primed by a subcutaneous injection of 30 I.U. of estrone[1] in 0.1 cc. of sesame oil. When the animals were not in actual use additional priming doses were given every 3 weeks. Animals which failed to show a

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[1] Estrone was generously provided for our work by Dr. George Harrop of the E. R. Squibb and Sons laboratory.

full estrous smear following the priming dose were discarded. In addition, small amounts of estrone dissolved in sesame oil were routinely applied to the vagina several days before the animals were to receive dried blood pellets, as a test for the maintenance of sensitivity. We found that approximately 90 per cent of our animals showed a threshold response to a total of 0.2 I.U. introduced into the vagina in 0.1 cc. of sesame oil on 2 successive days, a sensitivity which corresponds closely to that found by

TABLE 1. MENSTRUAL RECORDS OF SUBJECTS FROM WHOM BLOOD WAS OBTAINED FOR PREPARATION OF PELLETS

Subject No.	Av. Length of Cycle	Day Blood Taken	Weight, mg., and Number of Pellets		
			30	60	90
	days				
1	35	26	3	3	3
2	28	15	3	3	3
3	31	12	3	3	3
4a	28	10	3	3	3
4b	28	24	3	6	3

Albrieux for his animals. Vaginal smears were examined 48, 60 and 72 hours after the first application. Only animals which responded to this dosage of estrone were considered suitable for the pellet assay. The pellets to be tested were inserted deep into the vagina of the animal by means of a specially constructed trochar and canula. Complete absorption of the material retained within the vagina usually occurred within 24 hours. The animals were periodically examined for evidence of extrusion of the material. Vaginal smears were taken 48, 60 and 72 hours after pellet insertion. Three rats were used for pellets of similar weight and the results were to be considered positive if 2 of the 3 rats showed at least a threshold response.

Four young women with apparently normal menstrual cycles were used as subjects for this assay. The time at which blood samples were obtained for each test in relation to the menstrual cycle of the subject, and the number and weight of the pellets prepared are presented in table 1. An effort was made to obtain the blood during a period in the cycle which would be likely to correspond to the time of the midinterval and premenstrual peaks of the bimodal curve which is recognized as typical for urinary excretion of estrogen in normal women (8).

RESULTS

In no case was a definite positive vaginal response obtained in the spayed rats. Questionable smears were found in several instances; however, these doubtful smears were obtained from animals which had received the smallest pellets more often than from those receiving the largest, so that it is unlikely that they represented estrogenic stimulation.

During the course of this experiment there was only one instance of an entire pellet being extruded from the vagina. However, dried blood was found about the vaginal orifice of nearly all of the rats within several hours after insertion of the pellets, a finding which seemed to indicate that some portions of the pellets were being lost by vaginal extrusion. To determine if this were true, a number of pellets were so prepared that each contained 0.4 I.U. of estrone in the sesame oil. This is twice the amount found necessary for threshold stimulation when applied in oil directly to the vagina. In only 2 of 6 animals was a positive response obtained.

DISCUSSION

The failure to obtain any evidence of estrogenic stimulation is in all probability due to one of two reasons, or to both: *a*) not enough of the pellet is retained in the vagina to cause stimulation, or *b*) there is not sufficient estrogen normally present in the small amounts of blood which can be utilized in this method of assay to produce vaginal stimulation.

From our experience, the following objections to the pellet method of assay seem justified: 1. *Loss of material.* There is usually loss of at least some of the pellet before absorption. This occurs with the 30-mg. pellets as well as with those of larger size. 2. *Limitation to the amount of blood that can be tested.* There is a definite limitation in regard to the amount of blood that can be compressed into a pellet suitable for vaginal insertion. A 100-mg. pellet of dried blood is inserted with difficulty and undoubtedly with some trauma to the vaginal epithelium. Thus, this method is limited to testing the estrogenic content of less than one-half cc. of blood. 3. *The subjectivity of the determination of the end point.* A personal error is apt to occur in interpreting smears of the threshold type, where judgment of the stage of reaction is de-

pendent upon the worker's impression of the ratio of the various types of cells. 4. *The variability of the end point.* A number of investigators (9–13) have shown that because of the great individual variation among rats in their response to estrogens, the vaginal smear method of determining estrogenic stimulation is unreliable as a quantitative method unless large numbers of animals are used for each test. We have found that the same rat may react differently to the same dose of estrone (either injected or applied directly to the vagina in oil) at different times. Thus, an error may be introduced due to a fluctuating sensitivity in the vaginal response of individual animals to estrogenic substances. Also, as indicated by Deckert, *et al.* (13), the vaginal smear method depends upon the examination of a vaginal wash to determine the condition of the vaginal mucosa, and if this is not correctly interpreted by the character of the smear, another source of error is introduced. These workers obtained from castrated animals smears that contained more nucleated epithelial cells than leukocytes, with varying numbers of squamous cells; yet histological examination of the vaginal mucosa showed the tissue to be definitely of the castrate type. We have noted the same condition in castrated untreated animals showing smears that might be mistaken for a threshold response to estrogenic stimulation.

SUMMARY

In our experience with the biologic assay of blood estrogens by the intravaginal application of desiccated blood pellets in spayed rats, we were unable to obtain evidence of estrogenic stimulation with blood taken from young women with normal menstrual cycles at varying times during the cycle. The following objections to the method seem justified: 1. There is usually loss of some of the pellet from the vagina before absorption. 2. There is a definite limitation to the quantity of blood that can be compressed into a pellet suitable for vaginal insertion (less than one-half cc.). 3. The subjectivity of judging the end point (threshold smear) introduces the possibility of personal error. 4. The vaginal smear method of determining estrogenic stimulation is unreliable as a quantitative method unless large numbers of animals are used for each test.

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A Two and Six-Hour Pregnancy Test[1]

Preliminary Report

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AFTER their meritorious work on the detection of gonad-stimulating substances in the urine of pregnant women, Aschheim and Zondek proposed the first accurate test for pregnancy. The test is based upon the fact that, following the injection of urinary gonadotropins (which increase sharply 2 to 3 weeks after conception) into immature female mice, hemorrhagic follicles or "blutpunkten" occur in the ovaries. The A-Z test was soon modified by Friedman so that the isolated female rabbit was used for the test animal (2). The accuracy of the A-Z test or the Friedman modification is beyond doubt the highest of the many pregnancy diagnostic tests. Other pregnancy tests or modifications of the A-Z test have been offered simply because of the relative inconvenience of the original procedure and the Friedman modification. Although the Friedman test is the most widely accepted and used and is more applicable than the immature mouse test, nevertheless, it has some undesirable features, namely, the expense involved and difficulty of securing animals, especially during the present emergency, time necessary for completion of the test, inconvenience of isolation of the animals prior to use and the difficulty experienced by some technicians in the administration of the urine.

To surmount these difficulties, we have devised a 2 and 6-hour pregnancy test based on the 6-hour test advocated by Salmon, *et al.*, (3). The end-point of this test is dependent upon the hyperemic effect of urinary gonadotropin upon the ovarian stroma, corpus luteum and ovarian capsule. The weight and age of the animal used and end-point employed in this test are as follows: Immature female rats of 21 to 55 days of age may be used for the 2-hour test and female rats of any age are satisfactory for the 6-hour test. With reference to our procedure, we define an immature rat as one in which neither vaginal introitus nor corpus luteum formation has occurred. The criterion for a positive test for the 2 or 6-hour procedure in the immature rats is hyperemia of the ovarian capsule and tissue. The adult animal may be used only for the 6-hour test and may be of any age or weight providing senility or cessation of the ovarian cycles has not occurred. The use of the adult animal in the 6-hour test depends upon the vascularizing effect of pregnancy urine upon recently formed corpora lutea. It is important that only animals in diestrus or metestrus be used to allow for greater ease in reading the results. The corpora lutea of animals injected during estrus or proestrus do not show so characteristic an effect as those of animals injected during the metestrous or diestrus phase of the cycle. The corpora lutea in a positive test appear dark red or purplish in color.

The end points of these tests, although easily discernible require some experience before accurate readings may be made.

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PROCEDURE

Two-hour pregnancy test. Immature rats, as defined above, are injected intraperitoneally with 1.5 cc. of a morning sample of the urine. Divided doses of ¾ cc. are injected into both the right and left lower abdominal quadrants.[2] The animal is killed with ether two hours after injection of the urine and the appearance of the ovary and the ovarian capsule is noted. Hyperemia, as indicated by the reddish appearance of the ovary and capsule, is considered a positive reaction. Until one is able to distinguish between the normal appearance of the ovary which is white or pinkish in color and that of the ovary stimulated by pregnancy urine it is advisable to kill an uninjected animal (littermate preferred) at the same time as the test animal so that a comparison may be made.

Six-hour pregnancy test with immature rats. Two cc. of urine is administered by subcutaneous injection and the animal is killed 6 hours later. The end-point is similar to that observed for the 2-hour test.

Six-hour pregnancy test with adult rat. Before the urine is administered vaginal smears are taken and only those animals in metestrus or diestrus are used. Five cc. of urine is injected subcutaneously and the animal is killed 6 hours later. The recent corpora lutea of animals injected with pregnancy urine appear dark red to purple in color, and microscopically they show a marked degree of vascularization. Here again it is suggested that until one becomes adept in reading the tests, control animals in the same stage of the cycle should be killed, simultaneously, for comparison with the rats injected with urine. As an aid in reading these tests, we have found that a more definite reaction is observed 2 to 3 minutes after the animal has been killed and the viscera exposed for inspection. In addition, the reaction of the corpora lutea of the adult animals may be more easily interpreted when the ovaries are placed in formalin. The control ovaries and corpora lutea remain pale in color while those from animals injected with pregnancy urine turn dark brown or a decidedly darker shade than the controls after being placed in formalin. By means of this simple procedure a convenient set of standards may be prepared and kept indefinitely for comparison with the results obtained from unknown samples of urine.

[2] Two injections of ¾ cc. each were more effective than one injection of 1.5 cc. in producing the hyperemic effect upon the ovarian tissue. Whether this is due to a more complete absorption of urine from 2 injection areas or whether a large single injection resulted in poor distribution of the urine in the body cavity, thereby preventing rapid absorption of the urine we are unable to say. In any event the double injection procedure resulted in a sharper end-point.

RESULTS

Eighty-six samples of urine were examined and of these 48 were specimens from pregnant women and 38 from non-pregnant cases. Only 2 samples of the 85 yielded indecisive tests. However, when these 2 tests were repeated with specimens obtained 5 days later, strong positive reactions resulted. Since both of these urines became positive in less than 2 weeks after the date of the missed menstrual period, the first doubtful test was probably made before the gonadotropic titer had attained a detectible level. Two specimens of urine from pregnant patients were negative in 1 rat of the 3 used. In view of these results we found that the optimum number of rats to use for a diagnosis was 3, 2 for the 2-hour test and 1 for the 6-hour test. In this series there has been no case of a false positive test resulting from the urine of a non-pregnant individual. If necessary one rat may be used satisfactorily and if the test is positive there is no need to use additional animals. However, in instances of a negative test it is advisable to inject 2 more animals for confirmatory data. In addition we have found that although both the 2 and 6-hour tests may be read at 2 and 6 hours, respectively, they may also be observed almost anytime thereafter, but preferably not more than 36 to 48 hours after the injection of the urine. The test, during this period of time will remain positive or negative, whichever the case may be.

DISCUSSION

In order that a pregnancy test prove ideal *a*) it should be reliable; *b*) the test animal should be cheap and readily available; *c*) the time required for the test should be short and *d*) the test should be easily read. All these requirements are satisfied by the 2-hour test and

modification of the 6-hour procedure. The selection of animals for the 2-hour test is limited to those animals that have not experienced their initial estrous cycle. This permits of a rather wide selection and in our colony, animals from 21 to 55 days of age and from 30 to 100 gm. have proved satisfactory. Animals of almost any age group may be used for the 6-hour test. However, in the immature animals the test depends upon hyperemia of the ovary and ovarian capsule, while in the adult animals the vascularizing effect on the corpora lutea is the primary reaction.

We have tried the possibility of using similar end-points in other species of animals with the hope of making the test applicable to the usual laboratory animals. In preliminary experiments on a few test cases we have noticed that the 6-hour pregnancy test may be effectively employed by use of the immature female hamster (*C. auratus*). However, the data are still too few to warrant any definite conclusion.

From the academic point of view it is of interest to note the rapidity with which the urinary gonadotropin acts on the ovary. The state of hyperemia noted 2 hours after intraperitoneal injection and 6 hours after subcutaneous administration of the urine may represent the initial stage of reactivity of the tissue to hormone stimulation. Histological examination of sections of the positive ovaries have shown extensive hyperemia in the ovarian tissue of the immature animals and in the adult animals the corpora lutea were markedly congested by prominent vasodilatation. It is this increased vascularization of the corpora lutea that macroscopically gives them their red to deep purple appearance.

SUMMARY

A preliminary report is presented on a 2 and 6-hour pregnancy test employing rats of almost any age and weight. The two hour test is based upon the hyperemic effect of urinary gonadotropins upon the ovary and ovarian capsule noted 2 hours after intraperitoneal injection of 1.5 cc. of urine into immature female rats in divided doses of $\frac{3}{4}$ cc. each. The rats may be of any age (21–55 days) or weight (30–100 gm.) providing vaginal introitus or corpora lutea formation has not occurred. The 6-hour test may be performed on female rats of any age or weight. In the immature rats (similar to those used for the 2-hour test) the end reaction is the hyperemic effect of the urinary gonadotropin upon the ovary and ovarian capsule. The diagnostic feature in the adult animal is the vascularizing effect of pregnancy urine upon the corpora lutea of animals injected when in diestrus or metestrus. Of 33 specimens of urine from non-pregnant individuals, there was no instance of a false positive reaction. Only 2 of 48 samples of pregnancy urine gave an indecisive reaction, but the urines from these patients yielded strongly positive reactions 5 days later. In performing the test 3 rats are sufficient 2 for the 2-hour test and 1 for the 6-hour test. However, if necessary only one animal may be used if the test is positive, but in instances of a negative test it is advisable to inject at least 2 more animals for confirmatory data.

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Sublingual Use of Testosterone in 7 Cases of Hypogonadism: Report of 3 Congenital Eunuchoids Occurring in One Family

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THE effects of testosterone propionate by hypodermic injection, of free testosterone by pellet implantation, and of methyl testosterone orally in producing sexual development in hypogonadal cases have been reported by many observers. In1940, Anderson, Haymaker and Henderson (1) introduced the sublingual administration of desoxycorticosterone acetate in propylene glycol-alcohol. Other writers (2, 3, 4) have confirmed their successful results. Estradiol also has been used in this way by Salmon and Geist (5). In 1941, a trial of testosterone in propylene glycol-alcohol was suggested to me by Dr. Edward Henderson of the Schering Corporation, as a convenient method of administering testosterone and for comparison with clinical studies on testosterone pellet implantation. In 1942, Lisser, Escamilla and Curtis (6) reported successful sublingual therapy with testosterone, testosterone propionate and methyl testosterone dissolved in glycol.

This report deals primarily with the results of administration of testosterone in propylene glycol-alcohol sublingually in 6 cases of hypogonadism and 1 case of hypopituitarism. In the case of hypopituitarism (case 6) testosterone in propylene glycol-alcohol solution also was rubbed into the skin of one axilla and one side of the pubic area, demonstrating its local effect and confirming the results of a similar experiment by Albright (7) in a case of panhypopituitarism.

In cases 1, 2, 4 and 6 testosterone was given for the first time. The other patients, in whom maintenance doses of testosterone by pellet implantation, injection or oral administration had been established, were given sublingual testosterone for a comparison of its maintaining effect. The preparation was administered in sublingual doses of 1 or 2 drops, which the patient was instructed to hold there for 2 to 3 minutes without swallowing. When a larger amount was administered, the patient was instructed to take portions of it at convenient times throughout the day.

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REPORT OF CASES

Case 1 (Eunuchoid). A man (fig. 1, 4), aged 22 years' is 1 of 3 brothers similarly affected (cases 2 and 3). A fourth brother is normal. No family history of similar disorders was obtained.

At 16 years of age there appeared to be a slight spurt in sexual development which soon ceased. Occasional penile erections had occurred since the age of 9 years. Some sexual desire was noted. Occasional nocturnal emissions with little ejaculation occurred. His voice was high pitched, he had never shaved, and the hair was becoming gray prematurely.

The weight was 154 lb. the height 70.5 in. and the span 73⅜ inches. The blood pressure was 138 mm. Hg systolic and 90 diastolic. The hemoglobin was 82 per cent, and the erythrocytes numbered 4,400,000. The estimated bone age was 15.3 years (8); the ulnar and radial epiphyses were still open.

Treatment with testosterone in propylene glycol-alcohol was begun on April 21, 1941, 5 drops daily (4 to 5 mg.) of a solution containing 25 mg. of testosterone per cc. being given. On May 19, 1941, the dose was increased to 6 drops daily following which the patient noticed more frequent erections. On July 7, 1941, the dose was increased to 10 drops (8 to 9 mg.) daily and in September, 1941, it was increased to 12 drops (12 to 13 mg.). During this time the patient shaved once and noticed a lowering of his voice. In November, 1941, the dose was increased to 15 drops (approximately 25 mg.) daily of a solution containing 50 mg. per cc., which he continued to take until June 1, 1942, when it

FIG. 1, 2 and 3. Three brothers with hypogonadism before treatment. *Case 1* (*left*). Age 22 years, height 70.5 in., span 73⅜ in., and bone age 15.3 years. *Case 2* (*center*). Age 25 years, height 75¾ in., span 77¼ in., and bone age 17.3 years. *Case 3* (*right*). Age 27 years, height 68.5 in., span 72⅞ in., and bone age 17.3 years.

was reduced to 6 drops (10 mg.). At this time the height was 72⅛ in., the weight 139 lb., and the span 75 in. On July 6, 1942, he received anterior pituitary gonadotropic extract,[1] 100 units intramuscularly three times a week, and the testosterone was discontinued. On July 23, 1942, he noticed a decrease in the number of erections although there was no other gross change (fig. 5). The total growth during one year of treatment was 1⅜ in. while the estimated bone age increased from 15.3 to 16.9 years. There was no change in the size of the testes.

Case 2 (Eunuchoid). The second brother (fig. 2, 6) was 25 years of age. The weight was 180 lb., the height 75¾ in., the span 77¼ in., and the measurement from pubic region to floor, 42 in. The estimated bone age was 17.3 years. The testes were approximately 2.5 cm. in size, and the prostate gland was not felt. There was no facial or axillary hair and very little pubic hair. The voice was high pitched.

While this patient did not receive testosterone in propylene glycol-alcohol, the case is recorded for comparison with results obtained in his 2 brothers. Before coming to the Clinic he had received 1 cc. of chorionic and pituitary gonadotropic hormone[2] on alternating days for one month without benefit.

On Feb. 11, 1941, 150 mg. of testosterone[3] was implanted in the thigh, and shortly thereafter the patient noticed increased frequency of erections. The effect of the pellets lasted about 2 months. On July 14, 1941, 150 mg. was implanted; on September 3, 150 mg., and on Jan. 14, 1942, 150 mg. On January 14, the height was 76 in., and the weight 195 lb. In May, 1942, 300 mg. of testosterone was implanted. By this time the patient showed an increase in the growth of pubic and axillary hair and the voice was deeper. In July, 1942, 150 mg. was implanted. About this time the effect of the previous 300 mg. on penile erections had decreased. He received a total of 900 mg. of testosterone by pellet implantation.

In November, 1942, he had maintained the physical changes (fig. 7), although fewer erections occurred. The height was 76⅛ in., and the weight 190 lb. There was little change in the size of the testes. The total growth during treatment was ⅜ inches. The bone age increased from 17.3 years (Feb. 10, 1941) to 18.3 years (April 16, 1942).

Case 3 (Eunuchoid). The third brother (fig. 3) was 27 years of age. The height was 68.5 in., the weight 139 lb., and the span 72⅞ in. The estimated bone age was 17.3 years. The testes were soft and barely palpable, and no prostate was felt. This patient, like his

[1] Anterior pituitary extract (Praeloban) was supplied by the Winthrop Chemical Company, New York, New York.

[2] Anterior-pituitary-like and maturity factor, Ayerst, McKenna and Harrison, Montreal, Canada.

[3] Supplied by the Schering Corporation, Bloomfield, New Jersey.

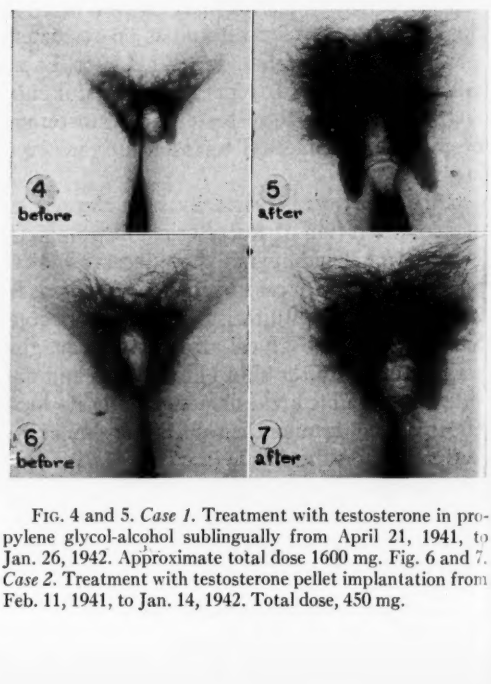

FIG. 4 and 5. *Case 1.* Treatment with testosterone in propylene glycol-alcohol sublingually from April 21, 1941, to Jan. 26, 1942. Approximate total dose 1600 mg. Fig. 6 and 7. *Case 2.* Treatment with testosterone pellet implantation from Feb. 11, 1941, to Jan. 14, 1942. Total dose, 450 mg.

brothers, had a high pitched voice. He had never shaved and had had no erections.

Treatment with testosterone in propylene glycol-alcohol was started at the same time as in case 1 (April 21, 1941), and continued in the same dose until August, 1942. Examination in March, 1943, showed the penis to be larger, the height to be 68⅞ in., and the span 73¼ in. The urinary androgens totalled 19.6 mg. per 24-hour specimen, the normal range being 13 to 30 mg. (9). The urinary test for anterior pituitary gonadotropins (FSH, prolan A)[4] was negative. No increase in testicular tissue was noted, but the voice had deepened and the pubic hair had increased. The hair on the legs became black and the facial hair increased, especially on the upper lip. The libido was normal, although the frequency of penile erections decreased after treatment was stopped. There has been no regression of genital development or of the pubic hair on cessation of therapy.

Comment. Because of the negative test for pituitary gonadotropins (FSH), the primary deficiency in this patient, as well as in his 2 brothers, would be considered by some to be a result of selective underfunction of the pituitary gland, that is, only the gonadotropic secretion being absent (9).

Case 4 (Operated cryptorchid). A boy, aged 13 years, first registered at the Clinic in May, 1940. An operation elsewhere in 1935 for undescended testes was followed by sepsis in the right operative wound. He had received 16,500 units of chorionic hormone elsewhere (antuitrin S) without noticeable effect. Analysis of the urine elsewhere showed 90 I.U. of estrogenic hormones, 90 I.U. of male sex hormone. The weight was 99¼ lb., and the height 58.5 in. The estimated bone age was 12.9 years. There was a small testis on the left and none on the right.

FIG. 8, 9 and 10. *Case 4.* Treatment with testosterone in propylene glycol-alcohol sublingually from May 25, 1941, to April 17, 1942. Approximate total dose, 5450 mg. 8. Before treatment. 9. Condition in April, 1942, before pellet implantation. 10. Condition in December, 1942.

On reëxamination in January, 1941, the weight was 107 lb. and the height was 57⅜ in. In April, 1941, the weight was 113 lb., and the height 57 $\frac{5}{16}$ in. Up to this time no change in the genitalia had occurred. In May, 1941 (fig. 8), testosterone in propylene glycol-alcohol, 4 drops daily of a solution containing 25 mg. per cc., was begun. In June, 1941, the dose was increased to 3 drops three times a day (approximately 8 mg.), and on November 15, it was doubled (15 mg.). This was continued until April, 1942 (fig. 9), when pellets containing 150 mg. of testosterone were implanted. The bone age was 14.9 years, pubic hair had developed, and the penis had quadrupled in size. Thus, he received

[4] The hormone of the anterior pituitary body capable of stimulating the gonads is found in the urine at the menopause, in castrates and certain eunuchoid patients wherein the testes or ovaries are thought to be incapable of stimulation. The present name applied to this hormone is follicle-stimulating hormone (FSH), formerly designated prolan A. It is quite possible that FSH is actually not the real stimulator of Leydig's cells which produce androgens but of the germinal or spermatogenic cells. A pituitary hormone analogous to the luteinizing hormone from the pituitary gland of the female (LH or prolan B) is postulated as the true pituitary androgenotropic principle (10), but a positive test for FSH in the urine is the best indication at present of the overproduction of gonadotropic hormones by the pituitary in an attempt to stimulate nonreactive or absent gonads.

about 4550 mg. of testosterone sublingually in 11 months. Beginning in August, 1942, methyl testosterone was administered for two months. The estimated bone age in January, 1943 (fig. 10), was 15.9 years. When last seen, the remaining testis appeared to have increased in size, being approximately 3 cm. in diameter.

Comment. When testosterone therapy was begun I realized that this boy might mature without treatment, and testosterone has been withheld without evidence of regression. Further observations and conclusions regarding the effect of testosterone on his growth will be reported later.

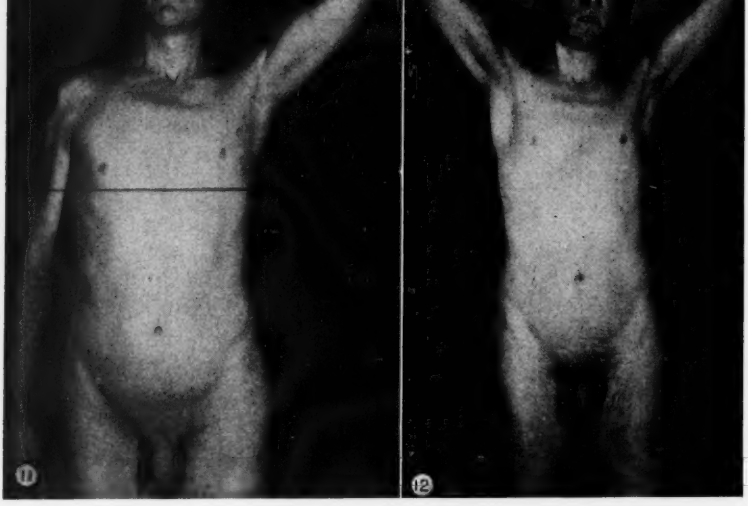

FIG. 11. *Case 6.* Before testosterone therapy, Sept. 25, 1942. Fig. 12. *Case 6.* After local inunction and sublingual therapy, March 8, 1943. Note growth of hair in left axilla, greater growth of hair on the left side of genital region and male distribution of hair.

Case 5 (Eunuchoid). A man, aged 21 years, had a height of 73.5 in., a weight of 197 lb., and a span of 74.5 in. Tests for urinary anterior pituitary gonadotropin (FSH) and estrogens were negative. The estimated bone age was 16.3 years. Throughout 1939 and 1940, he received 25 mg. of testosterone propionate by injection every fifth day. In March, 1940, genital development was comparable with that in cases 1 and 2 except for considerable increase in the size of the testes.

Beginning in March, 1941, he received testosterone in propylene glycol-alcohol, 6 drops daily (5 mg.) of a solution containing 25 mg. per cc. for three months. Previously he had taken an average of 35 mg. of testosterone propionate by injection per week, self-administered according to what he felt was necessary. Continuing with testosterone in propylene glycol-alcohol preparation, he maintained his status, that is, erections, voice and facial hair did not regress. From July, 1941, he took methyl testosterone, 10 mg. a day, 5 days a week, which appeared to maintain his condition to his own satisfaction.

In April, 1942, the height was 73⅞ in., the weight 184 lb., and the span 75 in. The epiphyses were well closed. The facial hair required shaving once or twice a week, axillary hair had appeared, and definite change in the body contour and growth had occurred.

Case 6 (Pituitary tumor with hypopituitarism). A man, aged 43 years, was operated upon in 1940 because of failing vision, the result of a chromophobe pituitary tumor. There was recurrence two years later with reoperation. On examination in September, 1942, he had no libido, no axillary or pubic hair and atrophic testes. The facial hair was sparse but bristly, and he shaved once or twice a week (fig. 11). Tests for urinary anterior pituitary gonadotropins and urinary androgens were not made. The metabolic rate before operation was −28 per cent, and the blood cholesterol was 298 mg. per cent.

The patient was advised to rub 2 drops of testosterone in propylene glycol-alcohol solution mixed with cold cream into the left axilla daily and on the left side of the pubic area. After one month no definite growth of hair was noted. The patient continued to apply testosterone to the skin and began to take 3 drops (10 mg.) sublingually each day. The total amount

administered sublingually was 500 mg., and the total amount rubbed into the skin was 500 mg. As a result, the patient felt generally improved, and had a slight return of libido. There was growth of hair in the left axillary and genital regions, greater on the left side, and growth of hair on the legs (fig. 12). No significant change in hemoglobin (67 per cent) or cholesterol (298 mg. %) occurred. The pubic hair returned according to its previous distribution, that is, of the male type.

Comment. I have not observed typical male distribution of pubic hair, that is, ascending and pointing toward the umbilicus, in any other patient in this group or in any other patient treated with testosterone. This, however, may have been due to insufficient dosage or duration of observation.

As stated before, Albright and co-workers (7) noted axillary growth of hair by local inunction in a case of panhypopituitarism. They believe that the adrenal cortex produces a hormone "similar to testosterone if not testosterone itself" and that either testicular or adrenal-derived androgen is capable of production of axillary or pubic hair. Since there is a difference between male and female pubic hair distribution, it may be that the male distribution including beard growth is the result of testicular-derived androgen superimposed upon adrenal-derived androgen such as found in the normal female.

If insufficient dosage is not the reason for the failure of development of the male escutcheon in eunuchoid patients, it is difficult to understand why administered testosterone does not adequately take the place of testosterone produced *in vivo*.

Case 7 (Traumatic bilateral testicular atrophy). A man, aged 22 years, ceased having erections and developed atrophy of both testes following an operation for ruptured appendix at 15 years of age. The patient is one of twins, being much less rugged and thinner than his brother who developed normally. The height was 71¼ in., the weight 117 lb., and the span 68 in. There was a moderate growth of body hair. The testes were small, 1 cm. or less in diameter. The epiphyses were closed. The voice was of medium pitch. He was timid and felt tired and weak.

Testosterone propionate, 50 mg. per week, was given by intramuscular injection beginning in July, 1940. He gained 11 pounds in two months and felt well. Frequent erections with an increase in the size of the penis occurred. The dose was reduced to 25 mg. per week and continued until January, 1941, when 100 mg. of testosterone in the form of pellets was implanted. Since he felt he had received more benefit from injections (which he well may have), in March he resumed injections of 25 mg. weekly for a month. Because of a loss of libido and a general decrease in his feeling of well being, he resumed injections in August and continued them until September, 1941, at which time he began taking 4 drops (6 mg.) daily of a solution containing 50 mg. of testosterone per cc. On this dose he maintained his progress; in fact, in two months he gained 10 more pounds (weight 138 lb.). The testes did not enlarge. The patient shaved about once a week, and his mental outlook was good.

Comment. In this patient, 4 drops (6 mg.) of a solution of testosterone in propylene glycol-alcohol maintained a status which had been satisfactory on 25 mg. testosterone propionate weekly by intramuscular injection (42 mg. a week as compared with 25 mg.).

Case 8 (Bilateral traumatic testicular atrophy). A colored man, aged 46 years, developed atrophy of the testes with loss of libido, hot flashes with profuse sweating, and mental depression following a bilateral hernia operation in 1937. The right testis was completely atrophied; the left was small but soft. The total urinary androgens averaged 3.82 mg. per 24 hours.

On a dosage of 50 mg. per week of testosterone propionate parenterally for 7 weeks, frequent erections occurred, but satisfactory intercourse was performed only occasionally. The mental depression, hot flashes and perspiration disappeared. The injections were stopped, and he was given testosterone in propylene glycol-alcohol solution, 10 drops (16 mg.) daily of a solution containing 50 mg. per cc. for 22 days, with gradual loss of effect. With 10 to 12 drops (33 mg.) daily of a solution containing 100 mg. per cc. for 21 days, he again felt much improved but not as well as when taking 50 mg. of testosterone propionate per week parenterally. This was continued for another month with about the same effect although the patient was not satisfied with his ability to perform sexual intercourse. Following the implantation of 6 pellets (75 mg. each), no further improvement occurred. Three weeks after implantation the total urinary androgens were 35 mg. per 24 hours. The addition of 20 mg. daily of methyl testosterone produced no further improvement in the patient's sexual capacity. He consequently became discouraged, but the depth of his depression was slight and hot flashes did not recur.

SUMMARY AND CONCLUSIONS

Three cases of eunuchoidism occurring in one family are reported. The effectiveness of testosterone in propylene glycol-alcohol administered sublingually was demonstrated. It would appear from these cases, although exact conclusions cannot be drawn, that to produce similar results in untreated eunuchoid cases two or

three times as much testosterone is needed sublingually as by pellet implantation.

For maintenance purposes, the sublingual administration of testosterone in propylene glycol-alcohol in eunuchoid patients compares favorably with intramuscular injection of testosterone propionate in oil in some cases but not in others.

The application of testosterone to the axillary and pubic areas was followed by local hair growth in a case of adult hypopituitarism from verified chromophobe tumor. The growth of pubic hair after the use of testosterone in eunuchoid patients usually does not have the typical male distribution. The growth of facial hair too is far from normal. Thus, testosterone does not serve as a complete substitute in the full development of all secondary characteristics. Impotence was not satisfactorily relieved.

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Thecoma and Hyperthecosis of the Ovary

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IN 1932 Loeffler and Priesel (1) described theco-cellular xanthofibroma as a new ovarian tumor, but such a new growth had already been described in 1926 by the Argentinian investigators Twaites-Lastra and Colillas (2). Approximately 50 cases have been reported since. This tumor differs from the common fibromas in that the cells contain phospholipid and cholesterol as indicated histologically by the characteristic appearance of fat in thecal cells (fig. 1). From the endocrinologic standpoint, such tumors differ from the fibromas because of the association of symptoms of sterility and menstrual disturbances with such thecomas.

I have observed (3) a case in which it was possible to show the direct transition of the theca interna of atretic follicles into tumor cells of this type. The patient, a female, aged 32 years had an extensive hypertrichosis, virilization and amenorrhea for 5 months. The tumor process involved both ovaries; no normal ovarian tissue could be found. The growth on the right was the size of a duck's egg, that on the left was as large as a hen's egg; both were hard in consistency and yellowish-white in color. Histologic sections showed a homogeneous structure interrupted only by a few small atretic follicles and a number of corpora albicantia. There were neither growing nor mature follicles nor recently formed corpora lutea. The tumor tissue was contiguous with the theca interna of some atretic follicles and was of identical histologic structure (fig. 2 and 3). The patient recovered promptly following the total removal of the right ovary and partial resection of the left ovary. The menses re-appeared immediately after operation and have been regular for 3 years. The virile habitus and voice, the facial hairgrowth and moustache disappeared within 8 weeks. The patient is now healthy and florid in countenance.

Some cases recorded in the literature are characterized by diffusely enlarged and hardened ovaries, together with a non-encapsulated tumor. In other cases there is only hardening of the ovaries. As the result of a long surgical experience, it seems to me that many ovaries which are removed on the assumption of fibrosis and cyst formation, are not fibro-cystic, but only diffusely enlarged ovaries, exhibiting a solid, homogeneous and hard constitution. Pregnancy in some cases has immediately followed resection.

The tumor, as is the case with common fibromata, is not definitely separated from the remaining normal tissue so that a new term has been introduced for this characteristic structure which is not that of a true fibroma but one corresponding to fibromatosis or, taking into consideration its relationship with the theca interna, to a thecomatosis. In the same sense we speak of an adenomyosis or endometriosis in order to avoid confusion with the adenomy*omas* and endometri*omas* which are true and sometimes very large new growths. The pituitary and thyroid glands show true adenomata, or else diffuse hyperplastic reactions, in association with clear-cut endocrine manifestations, *e.g.*, acromegaly, Cushing's syndrome (basophilism) and Graves' disease. In the same way that hyperthyreosis (hyperthyroidism) is amenable to surgical measures, hyperthecosis may also be relieved by operation, as this case demonstrates. The purpose of this report is to emphasize the importance of this condition which has not yet been properly recognized.

The physician should keep in mind the pos-

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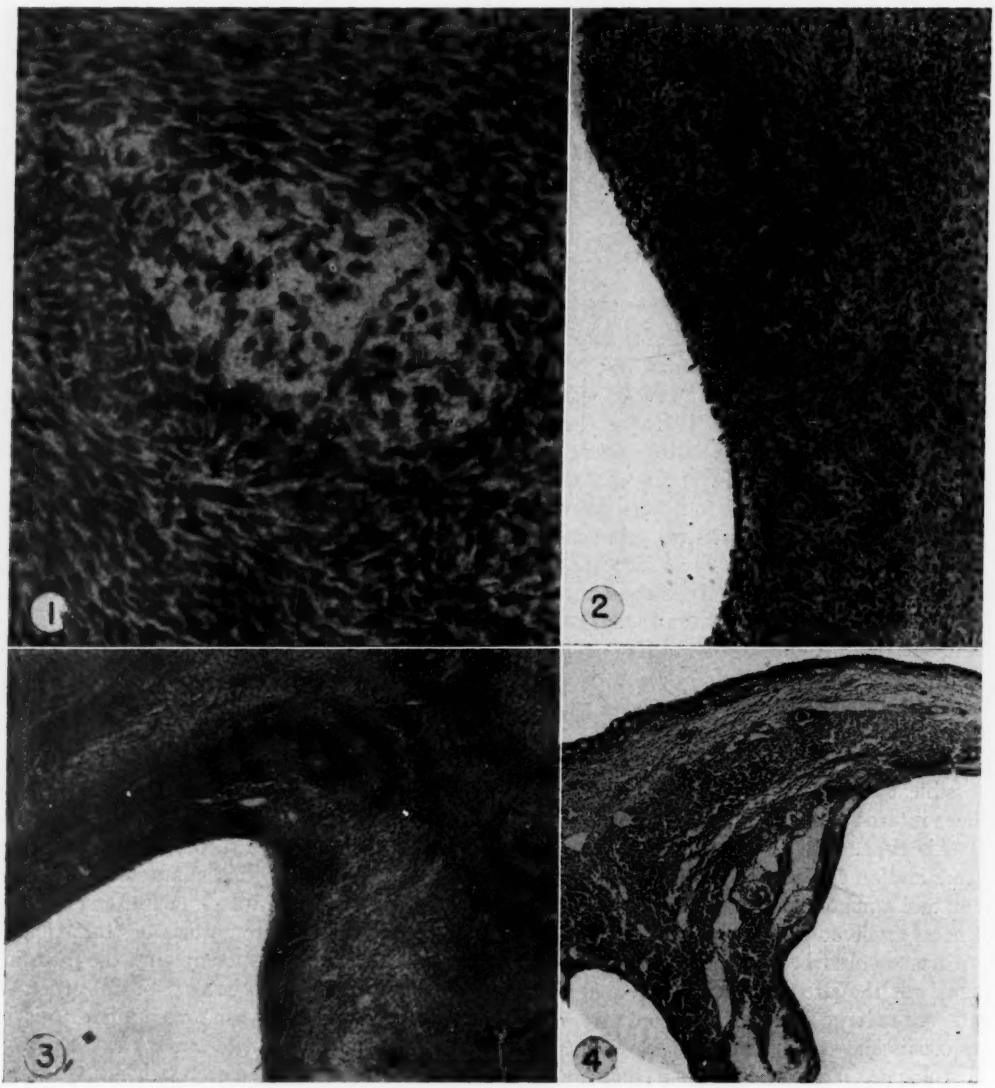

FIG. 1. Thecoma, typical structure. In the center, a group of 'foam cells.'
FIG. 2. Thecoma cells continuous with the theca interna of an atretic follicle (lumen in lower left corner).
FIG. 3. Atretic follicle with invading tumor cells originating from theca interna.
FIG. 4. Ovary of a newborn with diffuse hyperthecosis between two follicles (lumen below right and left corner).

sibility of thecoma and hyperthecosis in cases showing hard, enlarged and non-fibrocystic ovaries, associated with endocrine disturbances such as sterility, irregular menses, hypomenorrhea, amenorrhea or virilism. Medical treatment by means of androsterone or progesterone appears to be of little value in such cases of hormonal imbalance with unknown and variable hormone production. The same is true for roentgen-ray therapy, since the structure is connective tissue rather than epithelium. For these reasons surgical treatment seems to be indicated in the handling of these cases.

In addition to the case above, which came under observation at the Instituto de Endocrinologia de Montevideo, two more of this kind were diagnosed at the Instituto de Radiologia. The observations have been published

by Olascoaga and Blanco and it appears that this condition occurs more frequently than would be indicated by the lack of reference to it in the literature.

The evidence for the actual anatomic and physiologic existence of hyperthecosis is shown in the case report of a newborn which has been published in full (6). A thecoma can be ruled out (fig. 4). On the other hand, the hyperplasia and hypertrophy of both theca interna and externa might be considered a manifestation of the sex-hormonal crisis of the newborn. Five sites of such reactions are known already, *e.g.*, mammary gland, vulva and vagina, uterus, testicles and scrotum. As far as the ovary is concerned, only the growth, maturation and cystic degeneration of the follicles have been pointed out in this connection heretofore, no mention having been made of the changes in the theca externa. In our case (4) the proliferation of the theca involved the ovary throughout. I have observed hyperthecosis in the ovary of the newborn frequently since that time.

Finally, the question is raised whether or not tumors induced by injecting estrogen may be considered as either thecomas, hyperthecosis or xanthofibromas. Geist and Gaines (5) have experimentally produced thecomas by irradiating the ovaries of young animals.

From the purely theoretical standpoint, the fact that metastases have been observed from estrogen-induced tumors by Lipschütz (6) does not controvert a similar origin for hyperthecosis, since other benign new growths, *e.g.*, endometriosis, also show metastasis throughout the body.

SUMMARY

The actual presence of hyperthecosis in the ovary of the newborn and a clinical picture of hyperthecosis in the adult female have been described. The evidences for hyperthecosis are endocrine disturbances which are relieved by surgical measures (ovarian resection). From the theoretical as well as from the practical standpoint, the concept of hyperthecosis seems to be new and important.

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EDITORIAL

ENDOCRINE FACTORS IN RENAL FUNCTION

THE PRINCIPAL over-all aim of animal experimentation in the field of endocrinology is to determine with as much precision as may be the influence of each of the hormones upon all of the functions of the body. In the pursuit of this purpose the distribution of attention to the various bodily activities has been notably uneven. Among the major functions one of the most neglected has been the renal.

In a previous editorial[1] attention was called to some of the existing evidence that the volume and composition of the urine are influenced by androgens. More recently, the relationship of some other hormones to kidney function has been subject of a report from the Washington University School of Medicine at St. Louis.

Heinbecker and his colleagues[2] determined the diodrast and inulin clearances as a measure of renal blood flow, while kidney-tubule output was investigated by determining the maximum excretion of diodrast at high plasma levels. Trained female dogs were used as subjects.

It was observed that as early as a week after removal of the anterior hypophysis depression of renal functions supervened and persisted for considerable periods. The fact that the influence of the operation appeared so promptly was regarded as evidence that it was due to an immediate effect rather than being a secondary result of atrophic changes in other glands.

Complementary evidence was obtained by the injection of a commercial extract of ovine hypophyses. The diodrast and inulin clearances as well as the maximum diodrast output were increased. These effects were seen not only in normal dogs but also in those deprived of their hypophyses and in those subjected to an operation in which the pituitary stalk was cut and the tissues of the median eminence removed. The latter dogs displayed diabetes insipidus and obesity. In all, ten dogs were used for the hypophysis-extract studies. The results were apparently similar to the augmented urea clearance that has been reported to occur in acromegaly.

Similar determinations were made of the effects of thyroid "extract" (presumably desiccated thyroid substance) given orally in dosage of 0.1 gm. per kg. per day. This material also caused an increase in the renal functions, but less in degree than that of the hypophysis extract. These latter results throw some further light on the well-known diuretic effect of thyroid medication in clinical myxedema.

In further experiments in which extract of adrenal cortex was given subcutaneously, the results were less consistent.

The demonstrated effectiveness of anterior-lobe pituitary extract in increasing renal blood flow and augmenting the capacity of the renal tubules to transport diodrast adds further evidence that the kidneys are controlled to an important degree by the hypophysis.

R.G.H.

[1] Editorial: *J. Clin. Endocrinology* 3: 111. 1943.
[2] Heinbecker, P., Doris Rolf and H. L. White: *Am. J. Physiol.* 139: 543. 1943.

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: DANIEL A. MCGINTY. *Collaborators:* ISRAEL BRAM, JOHN C. DONALDSON, J. W. EVERETT, MURRAY B. GORDON, R. B. GREENBLATT, E. C. HAMBLEN, HANS O. HATERIUS, CHARLES W. HOOKER, R. G. HOSKINS, J. E. HOWARD, J. T. LEWIS, T. H. MCGAVACK, A. E. MEYER, MARY L. MILLER, C. C. PFEIFFER, DORIS PHELPS, J. P. PRATT, E. C. REIFENSTEIN, JR., BORIS B. RUBENSTEIN, PATRICIA H. SMITH, RUTH ST. JOHN, CHARLES W. TURNER, EMMERICH VON HAAM, HAROLD WOOSTER.

BOOK REVIEWS

CLENDENING, LOGAN, AND EDWARD H. HASHINGER.

Methods of treatment, 1033 pp. 8th ed. St. Louis, 1943. C. V. Mosby Co.

So well known is this book that little remains for the reviewer but to congratulate the authors that it has reached the current eighth edition.

Of special endocrine interest are three chapters devoted respectively to the use of glandular products, the treatment of diseases of metabolism, and of the endocrine glands. The first-mentioned chapter is developed upon a historical background and the physiologic rationale for the use of the standard products is elucidated. The chief indications for endocrine medication are discussed. The chapter should be very useful to the general practitioner though it is somewhat elementary for the specialist. Various commercial preparations are referred to by their trade names —a useful feature in a book of this type.

Most of the chapter on diseases of metabolism is devoted to the subject of diabetes but obesity is also included. The text is clear, readable, and adequate for its purpose. The chapter on endocrine diseases does about as well as could be expected in the 17 pages devoted to it.

The book as a whole is attractive in make-up and one which will continue its already well-attested usefulness.—*R.G.H.*

PAPANICOLAOU, G. N., AND H. F. TRAUT.

Diagnosis of Uterine Cancer by the Vaginal Smear. New York: The Commonwealth Fund, 1943.

For many years Papanicolaou has studied the cellular content of the vaginal smear and the great importance of his work in advancing the knowledge of endocrine physiology has long been recognized. In the course of his studies he found that cancer cells could be recognized in the human vaginal smear. This monograph presents a study of the abnormal cells characteristic of uterine carcinoma and the experience of the authors in the application of the vaginal smear technic to the diagnosis of cancer involving the female genital tract.

During the three years covered by the study, 3,014 women were intensively investigated. Between 7,000 and 10,000 vaginal smears were examined. Carcinoma involving the lower genital tract was found in 193 of the patients investigated. In 179 cases the primary lesion was in the uterus; in the remaining cases it was in the ovaries, bladder, urethra or vulva. There were 127 cases of cancer of the cervix, including seven very early types of squamous carcinoma. There were 53 cases of primary carcinoma of the fundus. Eight of these patients had very early superficial and circumscribed lesions. The vaginal smear findings revealed the presence of unsuspected lesions in 9 instances. In 2 cases, the vaginal smears revealed adenocarcinoma correctly when repeated curettage failed to do so because of the inaccessible location of the lesion. Failure to diagnose the malignancy from the vaginal smear findings occurred in only 11 of the 179 cases in which the cancer was primary in the uterus. Three of the failures were in postradiational cases and 6 others were in cases in which the lesion was adenoma malignum. Negative smears in the latter condition were attributed to failure of the neoplasm to shed its cells freely in the early stages of its growth. Malignant cells were detected in the vaginal smears in 13 of the 14 cases in which the primary lesion was extrauterine.

In the discussion of the significance of the findings with respect to the value of the vaginal smear as an accessory method for the diagnosis and study of uterine cancer, emphasis is placed upon the apparent reliability of the smear, in

the hands of careful workers, for the diagnosis of cancer of the uterus (except adenoma malignum), the usefulness of the technic for the study of very early stages of uterine cancer and its value as a means of bringing to the attention of the clinician the presence of unsuspected lesions.

In addition to a full description of the characteristics of the malignant cells found in the vaginal smears of the patients studied, the monograph includes a brief but adequate description of the epithelial cells of the vagina and cervix occurring in the vaginal fluid, the cellular contents of the vaginal fluid at different phases of the normal sex cycle and the effects of modified physiologic and of pathologic conditions on the cellular contents of the vaginal fluid. The method of obtaining smears is described and considerations relating to the correct use of the technic as a general diagnostic procedure are discussed. The text is beautifully illustrated with 11 colored plates showing the morphology and staining reaction of the various tyes of malignant cells described and of the cell types which occur in the vaginal fluid under different physiologic and pathologic conditions. The illustrations comprise, as was intended, an excellent and valuable atlas for comparison and study.

The book is clearly and concisely written and well printed. It is heartily recommended to the reader interested in the diagnosis of malignancy of the female genital tract or in the manifestations of endocrine activity in the human female. The worker who contemplates applying the vaginal smear technic to any gynecologic problem will find it especially valuable.—*D.P.*

ADRENALS

FINE, J., J. FISCHMANN AND H. A. FRANK.

The effect of adrenal cortical hormones in hemorrhage and shock. *Surgery* 12: 1. 1942.

I. The volume of circulating plasma and the total plasma protein of normal unanesthetized dogs was determined by the Evans' blue dye and specific gravity methods respectively. They were then subjected to a single massive hemorrhage (some 40 per cent of the total blood volume). Shock did not result. Four hours later the circulating plasma volume and total plasma protein were again determined and the compensatory mobilization of fluid and protein from extravascular depots calculated. Such observations were made in 41 dogs, grouped so as to show the effects of the following types of treatment administered immediately after the hemorrhage: 1) 2 cc. to 10 cc. (100–500 dog units) of cortin intravenously, 2) 150 cc. of physiologic saline solution distributed widely in the subcutaneous tissue, 3) cortin plus saline, 4) 150 cc. of plasma subcutaneously, distributed widely, 5) cortin plus plasma, 6) 10 to 15 mgm. of desoxycorticosterone acetate intravenously, 7) desoxycorticosterone acetate plus saline. Statistical analysis of the data revealed that the fluid-mobilizing capacity was not significantly increased by cortin or desoxycorticosterone acetate. Saline alone or plasma alone was equally effective. Significant increases were produced by cortin plus saline and cortin plus plasma, the former type of treatment being the more effective. Desoxycorticosterone acetate plus saline was no more effective than saline alone. The percentage recovery of lost circulating plasma protein was increased by cortin plus plasma but was not significantly increased by the other types of treatment. II. Dogs anesthetized with nembutal and subjected to acute blood volume loss of some 40 per cent uniformly and promptly went into shock, the criterion being a systolic blood pressure of 80 mm. Hg. or lower for 30 minutes or longer. In two dogs treated in this fashion, death occurred after about seven hours. The survival time was not prolonged by treatment with desoxycorticosterone acetate (3 experiments) or cortin (2 experiments). Recovery occurred after each of the following types of treatment administered intravenously 30 minutes or more after onset of shock: 1) 500 cc. saline solution (3 dogs); 2) 10 cc. cortin plus 500 cc. saline (5 dogs); 3) 200 cc. plasma (2 dogs); 4) 10 cc. cortin plus 200 cc. plasma (2 dogs). The return of the blood pressure to a sustained recovery level occurred almost immediately after cortin plus saline; with the other types of treatment 60 to 216 minutes was required for recovery. III. The experiments described in II were repeated, substituting for a single massive hemorrhage, successive withdrawals every 40 to 60 minutes of an estimated 10 per cent of the original total blood volume. A shock level of blood pressure was reached within two to five hours and death occurred within eight hours in hormone-treated as well as in untreated animals. In three out of four experiments with desoxycorticosterone acetate it was necessary to withdraw more blood to induce shock than in the control series. IV. In twelve dogs subjected to graded hemorrhage, plasma volume determinations were made at intervals during the period preceding the onset of shock. Two hours before bleeding began, three of the dogs were given desoxycorticosterone acetate intravenously and two were given cortin

intravenously. The remaining animals served as controls. Comparison of the three groups with respect to 1) the amount of plasma removed in order to induce irrecoverable shock levels of blood pressure, 2) the fluid volume compensation for a given volume of plasma loss, 3) the net loss of plasma volume at the time of onset of the shock level of pressure, 4) the extent of the compensatory mobilization of plasma proteins and 5) survival time revealed that the hormone-treated dogs had no greater tolerance to graded hemorrhage than did the control animals.

Conclusions: Desoxycorticosterone acetate is not an effective agent in the therapy of shock due to hemorrhage. Cortin given alone is not an effective agent in the therapy of shock. If it is combined with parenteral fluids it exerts some beneficial influence in certain circumstances.—*D.P.*

JACOBS, L.

Treatment of pertussis with vaccines and adrenal cortex extract. *Arch. Ped.* 60: 313. 1943.

The author followed the entire course of pertussis in 338 infants and children (age 1 month to 10 years) reporting to the clinics of Bellevue (N. Y.) Hospital. As controls, 108 received no specific therapy. Compared with these, patients treated with injections of pertussis antigen, pertussis vaccine and a mixture of equal parts of both, all showed definite improvement in the duration and severity of the disease. Similar improvement was obtained with triple typhoid vaccine, indicating that the benefits were due to non-specific stimulation of antibodies from foreign protein injection. A group of 15 patients received adrenal cortical extract, A.C.E. (Upjohn's), by injection (½ to 1 cc. every 2 to 3 days for 4 to 6 doses) during the severest epidemic season. The severity and duration of whooping cough in these patients was markedly diminished; viz.: coughing—10.2 weeks (control) to 3.9 weeks (A.C.E.); whooping—7.3 weeks (control) to 2.3 weeks (A.C.E.); and vomiting—5.8 weeks (control) to 2.2 weeks (A.C.E.). The sooner after the onset of symptoms that A.C.E. was given, the shorter the course of the disease. The author considered the action of A.C.E. non-specific and the mechanism unknown; he speculated that it may cause restoration of the normal electrolytic balance of the tissues, thus permitting more rapid and complete antibody formation.—*E.C.R., Jr.*

KOSTER, H., AND L. P. KASMAN.

Effect of desoxycorticosterone acetate in post-operative shock. *Arch. Surg.* 45: 272. 1942.

Two hundred patients operated on for conditions such that shock might reasonably be expected to develop during or after the operation were studied. One hundred alternately chosen were treated with desoxycorticosterone acetate and parenteral solution of sodium chloride. The mortality rate in the treated and control groups was used as the criterion of the effectiveness of the therapy, the term "shock" being assumed to denote an irreversible state of circulatory failure.

In the control and the treated group there were equal numbers of patients for the various operative procedures. All operations were performed with the patient under spinal anesthesia. The treated patients with acute conditions were given 5 cc. of adrenal cortical extract intramuscularly every six hours for the first forty-eight and then 5 mgm. of desoxycorticosterone acetate twice daily for four days and once daily subsequently for ten to fourteen days. All received 1,000 cc. of 5 per cent dextrose in physiologic solution of sodium chloride twice daily for the first four days. The other 94 patients in the treated group were given 5 mgm. of desoxycorticosterone acetate twice daily for from one to four days before operation and the same dose twice daily for the first four days postoperatively; after this only one injection a day was given up to the fourteenth day after operation. All of them received intravenous injections of 1,000 cc. of 5 per cent dextrose and saline solution twice a day before operation and for the first four days after operation. The 94 control patients were treated in exactly the same manner except that they received no desoxycorticosterone acetate or adrenal cortical extract. Transfusions and supplementary vitamin therapy were given to patients in both groups whenever it was believed the indication warranted it.

The effect of therapy on the operation and the convalescence in each case and the results in the control cases were tabulated. Among the 100 treated patients there was a mortality rate of 11 per cent and among the control patients, there was a mortality rate of 9 per cent. The authors point out that, even if reversed, this difference of percentages is not statistically significant. It was concluded that the results of the experiment afforded no evidence that desoxycorticosterone acetate can prevent or favorably influence post-operative shock as reflected by the mortality rate.—*D.P.*

ENDOCRINE GENERAL

BRAM, I.

The fat youngster. Guiding principles in treatment of 200 cases of alimentary obesity in patients under age 15. *Arch. Ped.* 60: 239. 1943.

The daily program includes: (1) diet (circa 1200 calories, with 3–4 glasses of milk, ¼ pound animal proteins, 1 egg, fresh vegetables and fruits in moderation); (2) fluids (limited to circa 6 glasses); (3) salt (restricted); (4) vitamin supplements (Brewer's yeast, 3 tablets); (5) exercise (in all but severe cases); (6) medications—a) to increase tissue oxidation, thyroid (grains 1/10 gradually increased to 1 to 2 grains), b) to reduce appetite, digitalis (1½ to 3 grains), benzedrine and propadrine (of doubtful value); and (7) psychotherapy (mutual confidence of doctor and patient, daily dietary diary by patient to maintain his interest). Of 200 cases (102 females, 98 males) with average age of 11.5 years and surplus weight of 28 pounds when beginning program, 184 (92%) have retained about normal weight for 2 to 5 years. Average reduction rate was 6 pounds per month; during first month about 1 pound a day. The author believes: (1) 95% of obese children are of alimentary type due to overeating because of unbridled appetite and parental indulgence; (2) functional imbalance of the developing endocrine system contributes to obesity; (3) eating is a major pleasure in many youngsters, but hearty exercise prevents obesity in most of them; (4) excess water drinking increases the tendency to corpulency; (5) basal metabolic rate is of little value in diagnosis in obesity, but tends to be on the minus side; (6) most important item in the reduction program is reduced food intake; and (7) after reduction, patients must be followed at regular frequent intervals for 3 to 5 years to guard against recurrence.—*E.C.R., Jr.*

BUEHL, C. C., AND S. I. PYLE.

The use of age at first appearance of three ossification centers in determining the skeletal status of children. *J. Ped.* 21: 335. 1942.

Onset of ossification was observed in three centers of the skeleton (distal epiphysis of the ulna, the first sesamoid of the thumb and the crest of the ilium) in order to test whether this stage of development is a reliable indicator of maturity. The age at which the epiphysis of the distal phalanx of the second finger fuses with its metaphysis was studied with a view to substituting it for iliac age. The observations were carried out on thirty boys and thirty girls. The mean ages for beginning ossification at the various areas were as follows:

Sex	Ulna years	Sesamoid years	Iliac years	Fusion of distal phalanx years
Male	7.6	12.6	14.3	14.7
Female	5.6	10.1	14.3	12.5

The mean age for the menarche in these girls was 12.7 years and in a comparative group of 100 girls, 12.9 years.

The authors conclude that the time of beginning ossification at the distal epiphyses of the ulna, the first sesamoid of the thumb and the crest of the ilium provide as reliable a measure of maturity as the concomitant mean total skeletal age in this group of children. Ossification in these centers appeared at an earlier age in girls than in boys. The ages at which ossification began in these three centers each correlated more closely with the menarcheal age than did the corresponding mean total skeletal ages in this group. (The skeletal ages of the hand and wrist, the elbow, the shoulder, the hip, the knee and foot and ankle are averaged and their mean recorded as the total skeletal age of the child.) Ossification began in the crest of the ilium within six months of the menarche in two-thirds of these girls. Fusion in the distal phalanx of the second finger also began near the menarche. It occurred shortly after the menarche in a majority of the group. It is suggested that onset of ossification in the crest of the ilium might possibly indicate a point in the maturation of the male which is comparable to that existing in the female at the time of the menarche.—*M.B.G.*

DAVIDOFF, E., E. C. REIFENSTEIN AND G. L. GOODSTONE.

The treatment of involutional psychoses with diethyl stilbestrol. *Am. J. Psychiat.* 99: 557. 1943.

A report is made of observations during a two-year period on the effect of large doses of estrogen in 60 women with involutional mental disorders. The estrogens were given for one to two months and usually then gradually withdrawn. Diethyl stilbestrol was given to 45 patients in descending doses, beginning at 4 mg. daily, tapered by 1 mg. weekly decrements to 2 mg. for the remainder of the treatment. To 15 patients was given estradiol benzoate in similarly diminishing dosage, starting with 1.66 mg.

(100,000 I.U.) four times and tapering to two times weekly. In the 9 patients from whom endometrial biopsies were obtained a marked estrogenic effect was noted. The work was controlled by 128 women who did not receive the estrogen. The two estrogens seemed about equally effective. In a control series of 128 cases 42% of the patients improved, whereas in the treated group 60% showed improvement. In those most severely affected the results were poor. Other psychoses occurring at the involutional period were not favorably influenced by estrogenic therapy. It is concluded that estrogenic therapy has a definite value in the treatment of involutional psychosis of the simple type.—*R.G.H.*

FLICKER, D. J.

Pubertas praecox in a female infant caused by ventricular cyst. *J. Nerv. & Ment. Dis.* 98: 42. 1943.

A fourth is added to the three which are stated to represent the only reported verified cases of pubertas praecox due to changes of the central nervous system. Breast enlargement was first noted at 18 months. In addition the patient showed retarded psycho-physical development, nocturnal eneuresis, strabismus and delayed closing of the frontal fontanel. Optic atrophy supervened. At operation in her fourth year a cyst of the third ventricle was found. Four years later she showed further enlargement of the breasts with adult vulva, hair distribution and uterus; the ovaries were palpable. Various pressure symptoms are also described.—*R.G.H.*

GLOBUS, J. H., J. M. ZUCKER AND J. M. RUBINSTEIN.

Tumors of the brain in children and in adolescents. A clinical and anatomic survey of 92 verified cases. *Am. J. Dis. Child.* 65: 604. 1943.

The series included 9 cases of pinealoma, and 6 cases of craniopharyngioma. In these there was a high incidence of manifestation of vegetative dysfunction expressed as retarded or accelerated development of secondary sex characters and as diabetes insipidus. These manifestations were thought to arise though anatomic and hence physiologic disturbance in the hypothalamus. One boy of 10 years with a craniopharyngioma and a normal pituitary had the obesity and sexual underdevelopment characteristic of Frohlich's syndrome.—*E.C.R., Jr.*

HODGES, R. G., W. M. SPERRY AND D. H. ANDERSON.

Serum cholesterol values for infants and children. *Am. J. Dis. Child.* 65: 858. 1943.

From a group of persons ranging in age from 1 month to 14 years, the authors eliminated those suffering from diabetes, nephrosis, hypothyroidism, obesity, malnutrition, infection, severe anemia, hepatic disease or other metabolic disorders; the remaining patients they considered normal. The mean value of the serum cholesterol concentration in 417 of this normal group was 205.7 ± 38 (standard deviation) mg. per 100 cc.; the mean value of the ratio of combined to free cholesterol in 344 of the normal group was 2.58 ± 0.29. In the excluded group, there was significant lowering of the total serum cholesterol with wasting diseases (78 patients) and severe anemia (18 patients); significant lowering of the ratio occurred with wasting diseases (78 patients), infection (25 patients), septicemia (9 patients), acute catarrhal jaundice (10 patients), and atresia of the bile ducts (15 patients), but not with severe anemia (18 patients). The total cholesterol level was not abnormal in 11 patients with non-obstructive cirrhosis of the Laennic type.—*E.C.R., Jr.*

HUNT, A. B., AND A. E. OSTERBERG.

Persistence of gonadotropin in dried pregnancy serum of human beings. *Proc. Staff Meet., Mayo Clin.* 17: 401. 1943.

Authors have used human pregnancy serum in the endocrine clinic for several years. This material may be dried in vacuum to a stable dry powder without loss of gonadotropic activity.—*D.A.M.*

LEWIS, R. C., A. M. DUVAL AND A. ILIFF.

Basal metabolism of normal boys and girls from two to twelve years old, inclusive. *Am. J. Dis. Child.* 65: 834. 1943.

In 1937, the authors reported the results of 637 determinations on 93 normal children in this age group. The present report extends their observations by 551 new determinations on 56 boys and 395 new determinations on 45 girls. The open circuit chamber method and the Carpenter-Haldane gas analysis apparatus were used. A statistical analysis of the data was made. Except in 4 isolated groups (exceptions of little importance), the mean values for calories per hour per square meter referred to age and for calories per

hour referred to weight, height, and surface area, respectively, did not differ significantly from those previously published. The basal metabolism exhibited the lowest degree of scatter about the mean, when expressed as calories per hour referred to weight and surface area, respectively, and as calories per hour per square meter referred to age. An intermediate degree of scatter is found for calories per hour referred to height and for calories per hour per centimeter referred to age, while the greatest dispersion is shown by calories per hour and calories per hour per kilogram, respectively referred to age. The mean values obtained by the authors are compared with the mean values found in the literature since 1936; the latter average from 0.3 to 7.3 per cent higher by the various methods of reference.—*E.C.R., Jr.*

LEWIS, R. C., A. M. DUVAL AND A. ILIFF.

Basal metabolism of normal children from thirteen to fifteen years old, inclusive. *Am. J. Dis. Child.* 65: 845. 1943.

This is a study of 90 determinations on 17 boys and 52 determinations on 13 girls. The means and the standard deviations from the means, together with their respective standard errors, and the coefficients of variation have been computed at convenient class intervals for calories per hour, calories per hour per square meter, calories per hour per centimeter and calories per hour per kilogram, respectively, in relation to age and for calories per hour in relation to weight, height and surface area, respectively. A comparison of the mean coefficients of variation for each of these methods of expressing the basal metabolism reveals that the data on boys show the lowest degree of scatter about the mean for calories per hour per square meter referred to age and for calories per hour referred to weight and surface area, respectively. An intermediate degree of dispersion is evidenced for calories per hour per centimeter referred to age and for calories per hour referred to height, while the greatest variation is found for calories per hour per kilogram for calories per hour, respectively, referred to age. These findings are essentially the same as those previously reported from this laboratory for children from 2 to 12 years old, inclusive. The mean values for the coefficient of variation for the girls differ somewhat from those for the boys. The lowest degree of dispersion is shown not only for calories per hour per square meter referred to age and calories per hour referred to weight and surface area, respectively, but also for calories per hour per centimeter referred to age. The value for calories per hour referred to age shows an intermediate rather than a large degree of scatter, while calories per hour referred to height and calories per hour per kilogram referred to age fall, as in the case of the boys, in the groups of intermediate and greatest dispersion, respectively. A detailed comparison has been made between the mean values obtained in this study for the basal metabolism of children 13, 14 and 15 years old and similar data reported in the literature.—*Authors' Summary.*

MÁRQUEZ, ANTONIO L.

The influence of testosterone on the state of hypertension in man. *Semana méd.* 50, I, 1180. 1943.

Report of 6 cases of moderate hypertension treated with an average of 10 mg. testosterone propionate 3 times weekly. The total doses varied between 50 and 200 mg. Symptomatic as well as objective improvement was obtained.—*A.E.M.*

POLATIN, P., AND H. SPOTNITZ.

Ambulatory insulin shock technique in the treatment of schizophrenia. *J. Nerv. & Ment. Dis.* 97: 5. 1943.

Forty-four patients with a definite diagnosis of schizophrenia were treated with insulin in dosages which gave definite physiological responses but not coma. The period of treatment ranged from one week to more than three years. Thirty-six patients (82%) showed definite clinical improvement, either slight or marked. Twenty-one patients (48%) showed a marked improvement or were considered recovered. Six out of eighteen patients (33%) who had a psychosis of less than one year's duration, were considered completely recovered. The duration of the treatment was dependent upon: (a) the duration of the psychosis before treatment and (b) the pre-psychotic personality of the patient. As a general rule, the patient had to be treated as long as his psychosis had existed prior to treatment in order to obtain a recovery response. When therapy was discontinued, approximately 33% of the patients who had manifested improvement, relapsed within two years following cessation of treatment. The tendency to relapse was believed to be due to an insufficiently long period of treatment and to the absence of intensive psychotherapy.—*R.G.H.*

SIMMONS, K., AND W. W. GREULICH.

Menarchal age and the height, weight and skeletal age of girls 7 to 17 years. *J. Ped.* 22: 518. 1943.

This is a statistical analysis of 1,339 annual examinations of 200 girls who are above average socio-economically. Correlations are made of the menarchal age; the standing height and weight; the skeletal age in 6 areas; and the annual increments in height, weight and skeletal age. In general, the authors found that the girl with early menarche as compared with the girl with late menarche: (1) has a greater standing height; (2) has a greater weight; (3) has greater weight for height; (4) has a greater skeletal age; (5) grows annually in height with the greatest acceleration, followed by the greatest deceleration; and (6) grows annually in weight with the greatest acceleration, followed by the greatest deceleration. In addition they found that: (1) the maximum increment in height occurs in the year of or preceding (but never after) menarche; (2) the maximum increments in weight tend to be spread over a two-year period; (3) skeletal age is more highly correlated with menarchal age than are standing height, weight, weight-height index, or annual increments in standing height; (4) the age at which menarche will occur can be detected by the skeletal age in advance of the menarche and in advance of the premenarchal acceleration in height and weight; and (5) rate of growth has no reliable relationship to terminal size.—*E.C.R., Jr.*

SNELLING, C. E.

Disturbed kidney function in the newborn infant associated with decreased calcium: phosphorus ratio. *J. Ped.* 22: 559. 1943.

The author reports the case histories of 11 newborn infants (1 to 11 days old) who exhibited the symptoms (twitching, convulsions, carpopedal spasm, cyanosis, etc.) of what has been called "tetany of the newborn infant." He found 11/11 of them to have hyperphosphatemia (5.5 to 11.0 mg.%); 9/11 to have hypocalcemia (7.3 to 9.2 mg.%); 11/11 to have non-protein nitrogen retention in the blood (40 to 160 mg.%); and 5/11 to have hypercreatininemia (2.75 to 7.6 mg.%). Two of 3 children who died showed renal lesions (hypoplastic kidneys; single kidney with hydronephrosis). The status of the parathyroid glands in these cases is not mentioned. The symptoms in all were relieved by the intravenous injection of calcium gluconate and glucose in saline. The author concludes that disturbed kidney function is the cause of this syndrome.—*E.C.R., Jr.*

GONADS

HAINES, W. H., AND S. MICELI.

Clinical observations on estrogenic therapy in prostatic and bladder carcinoma and benign prostatism. *Pennsylvania M. J.* 46: 1025. 1943.

The authors believe that two-thirds of all cases of prostate malignancy can be benefited by castration, estrogenic therapy, or both. Perineal biopsy is to be preferred to resection biopsy. The latter may miss the carcinomatous area. The question of optimum dosage of estrogenic substance is yet to be settled. Estrogenic action seems to be inhibited by infection. Estrogens relieve the symptoms of prostatism in many cases and also cause regression in size of the prostate. Estrogens help relieve the common symptoms of bladder malignancy in males. Estrogens seem to cause both clinical and cystoscopic improvement in mild (grade 1) carcinoma. Bladder malignancy may cause an increase in acid phosphatase.—*I.B.*

HUGGINS, C. B.

A summary of endocrine effects in advanced prostatic cancer. *Pennsylvania M. J.* 46: 1023. 1943.

In a series of 47 men with advanced prostatic cancer, it was found that 24 had elevation of acid and alkaline phosphatases, while in the other 23 the enzymes were in the normal ranges. By very frequent observation of the serum phosphatases of men with far-advanced prostatic cancer who had elevation of the serum phosphatases, it was found that decreasing the amount or the activity of the androgens by castration or by administration of estrogen (stilbestrol, 1 mg. daily) caused a decrease of serum acid phosphatase values, whereas androgen administration (testosterone propionate, 25 mg. daily) caused exacerbation of the serum phosphatase values and of the disease. The beneficial results of decreasing the androgens were not limited to the serum phosphatases. Forty-six men with advanced and metastatic cancer were treated by castration since October, 1939. When the cancer was discovered early, total perineal prostatectomy was done. Among the benefits following orchiectomy are an increased appetite and relief of pain, often observed within several days following castration. This results in a gain in weight and a return of the blood picture towards normal. Frequently, there is a decrease in size of the primary tumor, the prostate becoming smooth and soft and markedly diminished in size. Oftentimes, changes occur in

the bony metastases on roentgenographic examination. Usually the metastatic lesions undergo increased calcification within several months after orchiectomy. This is often followed by a stabilization in growth or by disappearance of the metastases. Other evidence of improvement which occurred in this series was, in 2 cases, disappearance of paralysis from metastasis in the central nervous system; in 2 other cases, disappearance of enlarged lymph nodes on the site of metastases. The onset of hot flashes similar to menopausal changes in women can be mitigated by estrogen administration. The improvement which occurs following orchiectomy far outweighs the undesirable effects. In the entire series of patients subjected to orchiectomy, there were 14 deaths, all in men with extensive metastases to bones. In 10, carcinomatosis was the principal cause of death, while in the others it was of secondary importance. From a clinical standpoint, 31 have had a sustained improvement lasting as long as 30 months; 9 have had a temporary improvement following castration. In 11 of the group of 21 patients operated upon 12 to 30 months ago, there has been significant improvement; these patients are free from symptoms, there has been complete or partial resolution of x-ray evidence of osseous metastases, and a decrease in size and in the stony consistency of the primary neoplasm on rectal examination. In 4 patients extensive osseous metastases have completely disappeared to radiographic examination. The author feels that bilateral orchiectomy is the method of choice as a basic treatment in advanced or metastatic prostatic cancer. —*I.B.*

KOZOLL, D. D.

Pseudohermaphroditism. *Arch. Surg.* 45: 578. 1942.

The numbers of reported cases of each of four types of hermaphrodites were brought up to date and two cases reported: the thirty-second case of a male pseudohermaphrodite with müllerian derivatives and the twenty-eighth case of a female pseudohermaphrodite. The genital tract of the eighteen-year-old male patient consisted of a normal penis, a prostate, a seminal vesicle, a scrotum, and a left testicle with its epididymis and a vas deferens. A well developed uterus with bilateral normal fallopian tubes and one broad ligament was present. Attached to the broad ligament was an intra-abdominal right testis and a right vas deferens and epididymis which did not communicate with the right testis. The external genitalia of the twenty-three-year-old female patient consisted of a penis with a glans and a prepuce, scrotal-like labial folds, a hypospadic external urethral meatus and a rudimentary vagina. The internal genitalia consisted solely of a rudimentary right half of the uterus, a right fallopian tube and a broad and a round ligament. Nothing was present in the left adnexa, and no gonadal tissue could be found on exploration.

Attention was called to the high incidence of inguinal hernias associated with hermaphroditism, hypogonadism and cryptorchidism and the advisability of intra-abdominal exploration of the hernial sac in cases of hernia associated with such deformities was emphasized.—*D.P.*

MCCAHEY, J. F.

Testosterone therapy in male hypogonadism. *Pennsylvania M. J.* 46: 1029. 1943.

Seven cases of male hypogonadism were treated with intragluteal injections of testosterone propionate. In all, the genitals were caused to develop. In all but one, the secondary sex characteristics were established. Two were sufficiently benefited generally to permit discontinuance of treatment. Treatment was also stopped in one because the maximum results did not warrant further therapy. One was relieved of marked psychoneurotic symptoms. Three were benefited psychically. The psychic benefits appeared to be connected with the induction of physical changes rather than with the establishment of the sex function. Subcutaneous implants of testosterone maintained the good effects of the injections for periods of 3 to 9 months. Four of these patients had previously been operated upon for undescended testes. It is suggested that some thought be given to conservation of testicular function in the decision as to appropriate measures in cryptorchidism.—*I.B.*

SCHONFELD, W. A.

Primary and secondary sexual characteristics. Study of their development in males from birth through maturity, with biometric study of penis and testes. *Am. J. Dis. Child.* 65: 535. 1943.

The author measured the range in size of the penis, testes and prostate and correlated these findings with the chronologic age and the maturation of secondary sex characteristics in 1500 males varying in age from 1 day to 25 years. The actual measurements and their percentile dis-

tributions are given. He found: (1) during first 2 years, only slight growth of testes and increase in circumference of penis; (2) growth of penis in length until 4 to 5 years of age; (3) no appreciable change thereafter until pubescence when there was a rapid spurt reaching maximum in the post-pubescent period; and (4) great variation (normal range 10 to 18 years) in age at onset of the pubescent spurt.—*E.C.R., Jr.*

HYPOPHYSIS

D'Ángelo Rodríguez, Aníbal, and José Manuel Puebla.

Hyperglucemic dyspituitarism. *Semana méd.* 50, I: 807 (1943).

The syndrome, which is illustrated by 6 case histories, is characterized by a moderately high glucemia (160) associated with a relative glucose resistance. The common symptoms are intense attacks of nausea, increase in weight, menstrual disturbances, decrease of libido, headache, anorexia, spastic constipation, edemas, increase in size of the liver and general nervousness. Hypercholesteremia is common but glucosuria is usually absent. The treatment consisted in high doses of estrogens or androgens either alone or alternating. The response was satisfactory and apparently lasting.—*A.E.M.*

Farber, J. E., F. J. Gustina and A. N. Postoloff.

Cushing's syndrome in children. Review of the literature and report of a case. *Am. J. Dis. Child.* 65: 593. 1943.

The patient, a white boy of 15 years, during the course of one year developed the typical "buffalo" type of obesity, hypertension, plethoric facies with hypertrichoris, purplish striae distensae, sexual precocity, generalized osteoporosis, weakness, backache, decreased tolerance for sugar, hypoproteinemia, hypochloremia, polycythemia. Perirenal pneumoroentgenograms outlined a large right adrenal carcinoma which was excised; patient died one day later. At autopsy: metastasis were found in the lungs, the left kidney, the adrenal vein and the inferior vena cava; the right adrenal gland was atrophic; the anterior pituitary showed no adenoma but the characteristic hyaline changes (Crooke) in the basophil cells; the thymus was not found; the pineal body contained a small involution cyst; the testicles showed active spermatogenesis and increased interstitial tissue; the thyroid contained increased connective tissue.

The authors tabulated with their own case 26 reported cases beginning before the age of 16. Of these 27, there was specific obesity in 26, hypertension in 25, osteoporosis in 18, sexual dystrophy (premature puberty and adolescence with onset of catemenia, usually followed later by amenorrhea in females and impotence in males) in all, hypertrichosis in all, lineae atrophicae in 23, polycythemia in 13, plethoric facies in 23. Weakness was present in 8 cases, but not mentioned in the others. There were 17 girls and 10 boys; no case has been reported in a negro child. In 12 cases there was an autopsy; in 4 others surgical exploration. In 8 patients, an adenoma of the pituitary gland was found; in 4, a tumor of the adrenal cortex; in 4, adrenal hypertrophy, and in 1, a thymic tumor. Four patients received irradiation which was said to have improved 3 of them. The authors concluded that the disease in children is characterized by premature sexual maturity.—*E.C.R., Jr.*

Mueller, C. B., and E. A. Graham.

Influence of hypophysectomy on the epithelization of wounds and on fibroplasia. *Arch. Surg.* 45: 534. 1942.

Skin over an area of two square centimeters on the right flank of eighteen normal and nineteen hypophysectomized young adult rats was resected; the animals were isolated; the wound was measured by means of cellophane and a wax pencil, and the area was calculated with a planimeter. There was no difference in the epithelization time of the wounds in the two series of animals. In the stomachs of twenty-nine normal and eighteen hypophysectomized rats, an incision 1 cm. long was made and sutured with No. 000 plain catgut. At varying intervals after operation, air was introduced into the stomach under measured pressure, and the pressure at which the wound broke was recorded (Harvey's method). The curves of healing of the two groups of animals were essentially similar in the phase of greatest fibroblastic activity and most rapid return of strength. During the initial lag phase the wounds of the hypophysectomized animals were slightly stronger than those of the normal animals. The results of the experiments were interpreted as indicating that hypophysectomy causes no change in the rate of epithelization of skin wounds or of fibroblastic repair of stomach wounds in the rat.—*D.P.*

PÁNCREAS

CANTILE, ENRIQUE, AND CARLOS FERNÁNDEZ SPERONI.

Endocrinopaties VI. Diabetes and menopause. *Semana méd.* 49, I: 499. 1942.

A case of a woman of 49 with a history of oligomenorrhea of long standing preceding menopause is described. She was obese and showed certain signs of pituitary dysfunction. The fasting blood sugar was 212 with a peak of 325 after ingestion of 100 gm. glucose. The blood urea was 0.033 gm. per cent. The treatment consisted of 50,000 units estradiol benzoate with 5 to 10 mg. testosterone propionate three times weekly. As a supplement, a short wave treatment of the pituitary region was given 3 times weekly. A marked reduction in weight was accompanied by a decrease of the blood sugar to 120 mg.—*A.E.M.*

EDELMAN, M. H., AND MIRIAM REINER.

Pentosuria associated with diabetes mellitus. *Arch. Int. Med.* 72: 31. 1943.

Two cases of essential pentosuria are presented and discussed. The coexistence of diabetes mellitus with essential pentosuria was discovered in one of these cases. Essential pentosuria and diabetes mellitus are distinct entities and seem to bear no relation to each other.—*I.B.*

HILDEBRAND, ALICE G., AND E. H. RYNEARSON.

Clinical experience with mixtures of protamine zinc and unmodified insulins. A preliminary report. *Arch. Int. Med.* 72: 37. 1943.

Generally speaking, mixtures of protamine zinc insulin and unmodified insulin have been shown to be capable of controlling to a satisfactory degree the levels of sugar in the blood of diabetic patients throughout the 24 hour period following their injection. With the exception of the period after breakfast, postprandial elevations of the blood sugar were minimal. The lag in the immediate hypoglycemic effect of the mixture does not constitute a serious objection to their use in cases of severe diabetes. The authors have not been able to demonstrate that any one ratio of the protamine zinc insulin to unmodified insulin gave an optimal effect, nor were they able to find an ideal mixture. However, ratios of protamine zinc insulin to unmodified insulin which lay between the 2 extremes of 1:1 and 1:5 gave the most satisfactory control in most instances; that is, mixtures in which the ratios of protamine zinc insulin to unmodified insulin were 1:2, 1:3, 1:4, 2:3 and 2:5 were capable of adequate control of the blood sugar during the 24 hours after their injection. The use of a mixture of equal parts of the 2 insulins resulted in blood sugar curves very similar to those obtained when the dose consisted of protamine zinc insulin alone. In such a mixture most of the unmodified insulin was in combination with the excess protamine. On the other hand, a mixture of 1 part of protamine zinc insulin to 5 parts of unmodified insulin resulted in a marked immediate hypoglycemic effect such as might be expected to follow the injection of a large dose of unmodified insulin alone. Evidently in such a mixture most of the unmodified insulin remained uncombined. The authors did not find that injection of the 2 types of insulins at separate sites gave as satisfactory control of the blood sugar level in the subsequent 24 hours as their injection together. Following their injection in separate sites, a marked immediate hypoglycemic effect was obtained, but smooth control of the level of the blood sugar during the remainder of the day and especially overnight did not result.—*I.B.*

THYMUS

SLOAN, H. E., JR.

The thymus in myasthenia gravis. With observations on the normal anatomy and histology of the thymus. *Surgery* 13: 154. 1943.

1) Gross and microscopic examination of 350 thymus glands, of which 150 were removed from patients dying suddenly, confirmed the well-known facts about the anatomy, histology, and involution of the thymus. 2) Of the 150 thymus glands examined in patients dying suddenly, fourteen were found to contain lymphoid follicles with germinal centers in the medulla. 3) It was impossible to set up significant standards of weight for the normal thymus since great variations were found in the weight, the amount of thymic tissue, and the amount of adipose tissue present. Hence, the weight of the thymus as an index of pathologic change was considered to have little value. The ten thymus glands in this series of patients with myasthenia gravis apparently were no larger than many normal glands.

4) Examination of ten thymus glands, none of which contained a tumor, removed at operation from patients with myasthenia gravis showed constant changes. a. In all there was some age involution. This varied from a slight degree of involution to marked separation of the thymic lobules by adipose tissue. b. In each case there

was an increase in the number of lymphocytes present in the thymus. This consisted of an infiltration of the medulla with large numbers of lymphocytes and usually a relative increase in the size and density of the cortex. The normal *sharp* differentiation between cortex and medulla was lost. c. In seven of the cases, abnormal numbers of lymphoid follicles with germinal centers were present in the medulla. d. There was no epithelial hyperplasia and no constant change in the character and number of the Hassall corpuscles. In six autopsied cases of myasthenia gravis examined which had not undergone operation there was no generalized lymphoid hyperplasia. The changes found in the thymus in myasthenia gravis were not part of such a generalized lymphoid hyperplasia.

5) The thymus was examined in individuals with Addison's disease, acromegaly, and hyperthyroidism. a. In seven individuals with Addison's disease, the thymus of three showed changes like those in myasthenia gravis. b. The thymus glands in five cases of acromegaly showed a striking lack of involution. In addition, two of these glands showed the changes found in myasthenia gravis. c. In twenty thymus glands examined from individuals with hyperthyroidism, eighteen showed a striking lack of involution. In addition, the changes found in myasthenia gravis were present in five.—*Author's Summary.*

THYROID

DAVIES, J. R.

Sporadic congenital obstructive goiter with recovery following operation in a thirteen-day-old infant. *J. Ped.* 22: 570, 1943.

The author describes a family with the following unusual events: (1) adenomatous goiter present for 11 years in mother; (2) first 3 children (males) normal at birth; (3) 4th and 5th children (males) born with congenital goiters which caused progressive tracheal obstruction and death within 1 month; (4) 6th child (first female) normal at birth; (5) 7th child (male) born with congenital goiter similar to 4th and 5th children, which was successfully removed surgically on the 12th post-natal day; (6) thyroid glands from the 4th and the 7th child on section hyperplastic (gland from 5th child not examined); and (7) first 3 children born 4 or less years before, all others 5 or more years after the appearance of the mother's goiter. The author concluded that the mother's thyroid functioned satisfactorily until the 4th pregnancy. He also reported another family in which: (1) the mother was of the hypothyroid type; (2) she bore six male children who died in infancy of convulsions (one had a congenital thyroid enlargement), (3) during the same interval she bore four female children three of whom were normal and one of whom died in utero at 6 months; (4) she then developed hyperthyroidism (tachycardia, tremor and exophthalmos) and had a partial thyroidectomy; and (5) thereafter, she bore two male and two female children all of whom were normal. The fact that the girls in these families escaped the fate of their brothers is cited as strong evidence of a hereditary factor in this congenital disorder.—*E.C.R., Jr.*

EMMETT, J. M., AND M. L. DREFUSS.

Early carcinoma in the hyperplastic thyroid. *Arch. Surg.* 45: 316. 1942.

A case of carcinoma of the thyroid arising in an adenoma in a hyperplastic gland is presented. The infrequent simultaneous occurrence of thyroid carcinoma and diffuse hyperplasia of the gland is discussed. The importance of miliary adenoma in the hyperplastic gland for the formation of carcinoma is stressed.—*Author's Summary.*

FRIEDMAN, H. H.

So-called "benign metastisizing goiter." Report of two cases with intracranial metastasis. *Arch. Surg.* 46: 377. 1943.

The literature relating to so-called "benign metastasizing goiter" was reviewed briefly. Pathogenesis, clinical features and treatment of the condition and arguments for and against regarding it as malignant were discussed. The findings in two cases illustrating the outstanding clinical and pathologic features were described in detail. The evidence presented was interpreted as indicating that: 1) In spite of the histologically benign appearance of the primary tumor or the metastases or both, the condition is to be regarded as malignant. 2) Intracranial neoplasms in patients with goiters should be considered metastatic until proved otherwise, even though the thyroid lesion appears to be insignificant.—*D.P.*

MCIVER, M. A.

Liver changes in hyperthyroidism. *Surgery* 12: 654. 1942.

The literature relating to liver changes in hyperthyroidism was reviewed and evaluated.

The following phases of the subject are discussed: 1) post-mortem date, 2) clinical evidence of liver damage in hyperthyroidism, 3) effect of thyroid crisis on the liver, 4) data from animal experiments, 5) increased susceptibility of the liver to injury under conditions of hyperthyroidism, and 6) therapeutic implications. Seventy-one references to the literature are listed in the bibliography.—*D.P.*

McIver, M. A., and Eleanor A. Winter.

Deleterious effects of anoxia on the liver of the hyperthyroid animal. *Arch. Surg.* 46: 171. 1943.

Male albino rats given injections of crystalline thyroxin for two to three weeks in doses of 0.1 mgm. daily in general remained in good condition, although they showed clinical signs of hyperthyroidism and when they were killed the hepatic glycogen level was found to be low. They showed no degenerative lesions of the liver. On exposure to atmosphere containing low concentrations of oxygen, in fourteen of seventeen hyperthyroid animals varying degrees of hepatic injury developed. There were nine deaths among the total of seventeen rats. In nine normal control rats similarly exposed to low concentrations of oxygen hepatic lesions did not develop. There were no deaths in this group. It was suggested that in some instances the acute lesions found in the livers of patients dying of hyperthyroidism may be the result of anoxia.—*Author's Summary.*

Moolten, R. R., and M. Bruger.

Exophthalmic goiter of the juvenile type. A survey of the literature on the familial aspects of this disease and a report of two additional cases. *Arch. Surg.* 45: 623. 1942.

The extensive literature on juvenile exophthalmic goiter is reviewed, and special emphasis is given to the familial aspects of this disease. Ninety-one cases of familial exophthalmic goiter in children are surveyed, and the possible familial source of the thyroid dyscrasia is indicated. In addition, two new cases occurring in children aged eleven and fifteen years, respectively, are presented; in the first of these, the mother had subtotal thyroidectomy for exophthalmic goiter, and in the second, partial thyroidectomy was done on the father for the same disorder.—*Author's Summary.*

Schneeberg, N. G., W. B. Likoff and D. R. Meranze.

An evaluation of the blood test for galactose tolerance in the diagnosis of hyperthyroidism. *Arch. Surg.* 46: 581. 1943.

The literature concerning the use of the blood test for galactose tolerance was reviewed and the authors' experience with the test summarized. Three hundred and four cases of thyrotoxicosis in which the test was performed were collected from the literature. Two hundred and eighty (92.1%) of the tests yielded positive results. In addition to the findings in hyperthyroidism, the technic, theoretical basis of the test, range of values in normal subjects, factors which may complicate the interpretation of the results, theories concerning the mechanism of the test and results of the test in disease conditions other than hyperthyroidism were dismissed. Forty heterogeneous diseases with abnormal galactose tolerance were listed. The following conclusions were drawn: 1) The test is useful in excluding the presence of hyperthyroidism in patients with hypermetabolism due to causes other than hyperthyroidism. 2) The test is unreliable in the diagnosis of hyperthyroidism in patients in whom thyroidectomy has previously been required for hyperthyroidism, in patients with decompensated cardiac disease and in patients suffering with peptic ulcer, hepatic insufficiency or Paget's disease. 3) The fact that the result of the test may be positive in any one of the heterogeneous disease entities presented and is rarely negative in the presence of hyperthyroidism emphasized the value of the test in excluding the diagnosis of hyperthyroidism rather than in establishing that diagnosis. 4) In instances in which an estimation of the basal metabolic rate cannot be performed, the blood galactose test may be substituted. 5) The mechanism of the altered galactose tolerance in hyperthyroidism does not appear to have been definitely established. Therefore we believe that Rowe's contention that galactose tolerance depends on a multiplicity of complex factors influencing galactose metabolism is the most fruitful concept thus far advanced in the interpretation of the test.—*D.P.*